FORAGING AND COLONY DYNAMICS OF RETICULITERMES SPP. (ISOPTERA: RHINOTERMITIDAE) IN GAINESVILLE, FLORIDA

By

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A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

1994

ACKNOWLEDGEMENTS

My deepest appreciation goes to my friend and husband, David H. Oi, also an entomologist, for his understanding throughout my work on this degree. I did not intend to force him through another Ph.D., but being able to discuss research problems and solutions with him as they arose helped me immensely. I thank him for understanding all the long, crazy hours, and late dinners. I'd also like to thank our families for their long distance support from Hawaii. Special love and appreciation go to my parents, Berg H. and Grace E. Fujimoto, who taught me to "stick to it."

Thanks go to Dr. "Chaos" Allen, who gave me much food for thought for future studies on termite population dynamics. Dr. Frank Slansky's nutritional ecology class changed my research philosophy. Dr. Barbara Thorne was always able to re-focus my research plan even from afar. Dr. Geoff Vining was an ever-patient statistician. Special thanks go to my Co-Chairs, Dr. Nan-Yao Su for support, advice, and emphasis on good science; and to Dr. Phil Koehler who opened new doors for me in the "opportunity-filled" world of extension. A hearty thanks go to the many friends made at the University of Florida, especially to the memory of Scott Ross Yocom, Ph.D., (Feb. 27, 1955-April 16, 1994)

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Abstract of Dissertation Presented to the Graduate School of the University of Florida in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

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August 1994

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The cryptic behavior of subterranean termites makes foraging dynamics difficult to study. The availability of Nile Blue A as a dye marker allowed the use of the weighted means, multiple mark-recapture method for population size, and foraging territory determination for five colonies in Gainesville, Florida. These colonies were typically smaller (12,000 to 2 million foragers) and covered smaller territories (up to 36 m^2) than those previously characterized in South Florida. Several colonies at any single site necessitated the search for other dye markers so adjacent colonies could be characterized simultaneously. Over twenty dye markers were evaluated. None had the longevity of Nile Blue A which persisted in termites in the field for up to ten months. However, several

short-term dyes were identified as useful in laboratory studies of behavior or feeding.

The ability of termites to consume cellulose from a variety of sources enhances its pest status. Termites were shown to prefer cardboard over filter paper in a binary choice test. Termites were able to discriminate between the cardboard and filter paper even when adjacent to each other, and differences in consumption were greater when cardboard and filter paper were separated at all time intervals ($p<0.05$). When termites were allowed access to equivalent food sources, both filter paper pairs and cardboard pairs were fed on differentially for all time intervals ($p<0.0001$).

Evidence that termites are generalist cellulose feeders can be exploited in current bait technology. The IGR, pyriproxyfen, was incorporated into cardboard, a general cellulose matrix. In the laboratory, R. virginicus which fed on pyriproxyfen-impregnated cardboard for four weeks at 100 and 150 ppm, significantly increased in soldier intercaste proportion. Experiments on the effect of various soldier proportions on termite survival, indicated that termite cohorts which sustained $\geq$20% soldiers died at a rate significantly faster ($p<0.01$) than termite cohorts which sustained the normal soldier proportions of 1 to 3%. A field test on an aerial infestation suggested that R. virginicus was suppressed and possibly eliminated after 3 weeks of feeding on cardboard impregnated with pyriproxyfen.

CHAPTER 1
INTRODUCTION

Termites, order Isoptera, are estimated to be over 100 million years old, possibly even 200 million years old (Anonymous 1993). Termites are eusocial, like ants, bees, and some wasps of order Hymenoptera, but unlike the haploid Hymenoptera, termites are diploid (Wilson 1971). Eusociality is characterized by the presence of cooperative brood care, overlapping generations, and reproductive division of labor (Matthews & Matthews 1978). Subterranean termites (family Rhinotermitidae) in a colony are divided into three castes: reproductives, soldiers, and workers (Krishna 1969). Functional reproductives can be either primary or supplementary. Primary reproductives are heavily sclerotized, macropterous, and usually present as a pair (Krishna 1969). Supplementaries are brachypterous or apterous, lightly sclerotized, and are often found in groups (Krishna 1969). Soldiers belong to the defensive caste. Workers forage for food, tend the dependent castes, and can be combative during colony defense.

Termites are cryptobiotic, soft-bodied insects, seemingly easy prey for man (Logan 1992), other mammals, and arthropods. However, they are equipped with many unique defensive mechanisms, not limited to the soldier

caste, including gut-exploding, mandibular slashing, and chemical secretions (Prestwich 1984). Soldiers are generally considered the defensive caste, but workers are also known to be combative in inter- and intraspecific interactions with other termites (Howick & Creffield 1980, Thorne 1982, Clément 1986, Su & Scheffrahn 1988a, Thorne & Haverty 1991). Agonistic behavior in termites has been one method used to investigate colony territoriality (Nel 1968, Binder 1988, Jones & Trosset 1991), when mark-recapture techniques were not used (see Chapter 3). Attempts have been made to correlate agonistic behavior with hydrocarbon phenotypes, under the hypothesis that these phenotypes serve as a recognition cue (Haverty & Thorne 1989, Bagneres et al. 1991, Su & Haverty 1991).

Before mark-recapture techniques were refined for use in colony demographic studies, the foraging dynamics of subterranean termites was largely unknown (see Chapter 2). Destructive sampling methods produced population estimates for Reticulitermes spp. of >100,000 (Pickens 1934) to 244,445 (Howard et al. 1982). Destructive sampling includes direct counts, baiting, and core sampling of the termite infested soil, but because of the subterranean nature of these termites, the location of the nest and the extent of the gallery system was largely unknown, making accurate estimates difficult to obtain. Mark-recapture is considered a non-destructive sampling method. These methods produce population estimates based on the ratio of marked to unmarked individuals over a given period of time, thus knowledge of the gallery

system and nest location is not necessary. One disadvantage to mark-recapture is that it can only estimate the foraging population, not the total population in termites, but the technique does leave the colony intact for long-term studies. Population size and territory estimates with mark-recapture reveals that colonies of Reticulitermes flavipes (Kollar) and Coptotermes formosanus Shiraki can exceed 5 million (Su et al. 1993) and 10 million individuals (Tamashiro et al. 1980, Yates & Tamashiro 1990) respectively, covering territories as large as 2,361 m^2 (Su et al. 1993) and 3,571 m^2 (Su & Scheffrahn 1988b), respectively. The huge colonies and territories of these termites make control a formidable task.

Of over 2,200 known species of termite (Weenser 1965), there are only 45 species in the United States, of which only five are considered to be economically important (Su & Scheffrahn 1990a). The termites which are considered urban pests, cost consumers over $1 billion annually in repair or control costs. Factors which contributed to the rise of termites as major urban pests are described below.

The Rise of Termites as a Pest

In 1876, Hagen (1876) wrote an article for the American Naturalist on "The Probable Danger of White Ants." He incorrectly predicted that the termites would retreat with advancing civilization. Although the prediction was incorrect, his article would indicate that the potential destructive capabilities of termites were contemplated at least 116 years ago. (As a frame of reference, the U. S.

Congress passed the Morrill Act in 1862, which started the Land Grant College system. By 1876, the Civil War had ended and people were expanding westward. In 1887, Congress enacted the Hatch Act which started the Experiment Stations.)

The Journal of Economic Entomology, which began in 1908, contained only one descriptive article about termites, in 1911, on the "California Redwood Attacked by Termes lugifugus Rossi" (Parker 1911). The next articles to reference termites in the Journal of Economic Entomology appeared two years later entitled: "The Insects Affecting Sugar Cane in Porto Rico [sic]," the termite was Termes morio Lath. (Van Dine 1913) and "White Ants, Historical" (Weiss 1913). Another two years passed before a half column snippet entitled: "A Cricket Predaceous on the Termite" (McColloch 1915) was published. (As a frame of reference, in 1914, Congress enacted the Smith-Lever Act that established the Extension Service which is so instrumental in public education of urban entomology.)

These articles were hardly a distinguished introduction for termites from the premiere journal for economically important insects. Judging by the focus of the journal articles, clearly maintaining the food supply for a quickly expanding population was of greater importance than termites infesting houses. Another reason termites may not have been given their due attention is that they were not infesting houses built of hardwood timber which was plentiful and used for construction post-Civil War. Once the hardwoods were depleted due to the

demand for housing for an expanding population, houses were constructed of second growth timber which was less dense and more prone to termite attack (Snyder 1936).

Termites were not considered pests until the normally cryptic subterranean termites were noticed by the general public until their homes were infested. In 1916, the USDA, reported 37 requests for information of termite biology and control; 15 requests came from Washington D. C. In 1917, there were 47 requests for termite information, 9 in Washington D. C. alone. In 1918, there were 39 requests, 13 from Washington; and in 1919, there were 42 requests, 12 from Washington alone (Banks & Snyder 1920). Similar trends in increasing termite damage were noticed in New York where there was one report of infestation in 1932; but in 1933, the numbers increased to greater than 12. In 1934, substantially more infestations were reported, although no numbers were given (Sanders 1935).

Early Efforts at Control

During the period of 1900-1935, efforts to quell problems of termite infestations increased. In 1898, Dr. Karl Henrich Wolman developed the Wolman salts as a wood preservative. In conjunction with the Antimite Company, Wolman also developed a product for termite control which used the Wolman salts. Records go as far back as 1925 to indicate that the Antimite Company was one of the first companies exclusively "engaged in helping the exterminator to solve the problems of termite control work" (Kreer 1934, p. 14).

In 1900, the Bureau of Entomology and Plant Quarantine was conducting follow up studies on termites control research. By 1911, more wood preservatives were proposed and experiments were conducted at Falls Church, Virginia, to determine the most effective preservatives and application methods. By 1924, these experiments had expanded to sites at the Canal Zone, Panama; and by 1928, the International Termite Exposure Test was formed which increased test sites to numerous tropical countries where termite damage was serious (Snyder 1935).

The use of wood preservatives was not the only method of protection against termites being investigated. Changes in city building codes were being proposed and enforced. In 1923, Burlington, Iowa, was the first to dictate a building code to prevent termite infestation (Snyder 1935). In 1927, the Pacific Code Building Officials followed suit by adopting the Bureau of Entomology and Plant Quarantine recommendations for termite damage prevention (Snyder 1935). In 1928, the City of Honolulu, Hawaii, adopted similar provisions (Snyder 1935). During this time, the idea of soil termiticides also surfaced.

Soil Treatments

Trapping and baiting, spraying infested timbers, and the injection of poison dusts for subterranean termite control were all tried and considered failures (Turner 1941). Early investigators also knew that fumigation and heat treatments were not effective for subterranean termite control because the

treatments were not residual (Turner 1941). Therefore, the only tool left to their disposal was the termiticide barrier method.

The concept of ground treatments as a barrier method arose in the 1930's. Investigators observed that chemical barriers were mainly to prevent the penetration of the treated layer of soil by termites (Randall & Doody 1934a). They also observed that the thickness of the treated soil layer appeared to be more important than the percentage of the termiticide; thus, soil treatments had to be thick enough so that the barrier would not be broken by ordinary disturbances (Randall & Doody 1934a). Trenching was also a common method of remedial termite control (Randall & Doody 1934a). The first termite control work was conducted in San Joaquin County, California (Randall & Doody 1934a). Sodium arsenate was sprayed under infested homes at rates of 6% at 20-50 gallons/100 square feet and 2% at 50-100 gallons/100 square feet which is as high as three times the amount of active ingredient for most currently registered soil termiticides and a rate 5-10 times higher than the labeled rate of 1 gallon/10 square feet.

Not until 1927, was there a concerted effort to organize researchers in the termite control area. This effort produced the Termite Investigation Committee (Brown 1934). Compounds tested by the committee for use as soil termiticides were Borax and magnesium fluosilicate at 5% and 10% (1 gallon/10 square feet). Other solutions to be tested were sodium chloride (strong solution), ammonium fluoride, sodium fluoride, sodium fluosilicate, and a kerosene

emulsion with 30 ml sodium arsenate per gallon (Brown 1934). The "researchers" for these experiments were not university scientists, but pest control operators. They also attempted soil fumigation with compounds such as carbon bisulfide, carbon tetrachloride, and other liquid fumigants such as paradichlorobenzene (PBD). Minus PDB, these treatments left little residual (Brown 1934).

Pest control companies began to promote proprietary products; however, without benefit of the current label requirements instituted by the EPA, under Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), there appeared to be little quality control. For example, the Antimite company promoted a dry salt of sodium fluoride, dinitrophenol, and sodium arsenate to be mixed at a rate of 10 lbs./30 gallons of water and applied by "generous drenching" (Randall & Doody 1934a, p.512). The American Fluoride Corporation of New York sold Fluorex V, a 5% sodium fluosilicate solution, and Fluorex S, a 10% magnesium base fluosilicate solution (Randall & Doody 1934a). The E. L. Bruce Company of Memphis, Tennessee, produced Terminix, a mixture of orthodichlorobenzene with "toxic solvents" and "toxic salts" (Randall & Doody 1934a, p.512). The E. L. Bruce Company was probably one of the first companies to issue a five year guarantee for their treatment and inspection service, but they required that wood be completely isolated from ground contact. None of these compounds had been tested by the Committee.

The Committee concluded that ground treatments should not be depended upon as a fundamental method of termite control (Randall & Doody 1934a), and soil treatments were not considered a permanent remedy (Snyder 1935). Proper construction and the insulation of all unprotected wood from ground contact was generally conceded to be the best method of preventing damage by subterranean termites (Randall & Doody 1934a). Despite this warning, the production and use of soil termiticides flourished. The next pesticides tested after inorganic salts are listed in Table 1-1. None of these compounds is currently registered for termite control.

Table 1-1. Chronological list of chemicals tested as soil termiticides and corresponding reference from the first published article in the Journal of Economic Entomology on the topic to 1970

Reference	Chemicals Tested
Hockenyos 1939	orthodichlorobenzene, trichlorobenzene, crude dichlorpentane, crude diamyl phenol
Smith 1939	diphenylamine
St. George 1944	DDT
Kowal & St. George 1948	lead arsenate, sodium fluosilicate, cryolite, phenothiazine, diphenylamine, pthalonitrile, trichlorobenzene, orthochlorobenzene, DDT, creosote
Hetrick 1950, 1952, 1957	DDT, dichlorodiphenyldichloroethane, methoxychlor, lindane, chlordane, pentachlorophenol (PCP), sodium pentachlorophenate (SPP), toxaphene, parathion

Table 1-1 (cont.)

Reference	Chemicals Tested
Ebeling & Pence 1958	DDT, chlordane, toxaphene, heptachlor, lindane, aldrin, dieldrin, parathion, malathion, diazinon, PCP, SPP, sodium arsenate
Bess et al. 1966, Bess & Hylin 1970	aldrin, dieldrin, chlordane, DDT, heptachlor, lindane, and sodium arsenate

The last of the cyclodienes, chlordane, was withdrawn by the manufacturer in 1987. The loss of chlordane was and is still mourned by PCO's because this termiticide had a reputation for long residual protection and was economical to use compared with pyrethroids. After 24 years of field testing in Hawaii, chlordane (1.0%) caused 88-94% mortality in Formosan subterranean termites after exposure for five days, and termites were able to forage through 75-100% of the core sample (4 cm long) of three different substrates brought back to the laboratory (Grace et al. 1993b). By year 28, these figures declined to 54-83% mortality, and penetration increased to 93-100%; and by year 33, mortality had dropped to 7-12% with 100% penetration (Grace et al. 1993b).

Soil termiticides, in 1994, contain one of the following active ingredients: bifenthrin, chlorpyrifos, cyfluthrin, cypermethrin, fenvalerate, and permethrin. With the exception of chlorpyrifos which is an organophosphate, these active ingredients are predominantly pyrethroids. Currently registered and potentially new soil termiticides continue to be tested (Su et al. 1987, Su & Scheffrahn 1990b, 1993a; Grace et al. 1993b) because certain states with high incidence of

subterranean termite infestation have mandated that structures be pretreated with a chemical barrier before the foundation is set as a protective measure (Florida Statutes 1991).

Termiticides are the only pesticides required by the EPA to show five years of efficacy in field trials (Kard & Mauldin 1990), while the law requires only safety data for other pesticides. Currently labeled termiticides are shown to be toxic or repellent, thus, effective in preventing penetration by termites in laboratory studies (Su & Scheffrahn 1990b), but PCO's claim otherwise. Termite control is a high-stake business in this litigious society. The trend in soil termiticides has gone away from highly water soluble inorganic salts, such as sodium arsenate, to highly insoluble compounds, in an effort to protect our water sources from accidental run-off. Mammalian toxicity has decreased from an LD_{50} of 50 mg/kg for sodium arsenate, to LD_{50}'s of > 3000 mg/kg for pyrethroids (Windholtz et al. 1983). Legislative control has also made it virtually impossible for the small pest control operator to experiment with home remedies for termite control. Tighter label restrictions help insure termite control quality.

Despite these facts, re-treatments occur at the alarming rate of 3-5 years (Tamashiro et al. 1990) which introduces significant amounts of a residual compound into the environment. Concerns for environmentally friendly solutions to termite control have forced researchers to re-focus efforts at studying the much neglected behavior and biology of this insect. Basaltic rock barriers, borates, and baits have resurfaced as ideas worthy of investigation.

Basaltic Barriers, Borates, and Baits

Ebeling & Pence (1957) first observed that R. hesperus would not penetrate particles of certain sizes. Like chemical barriers, the principle behind basaltic rock is to create a continuous physical barrier to exclude termites from entering a structure. Particle sizes must be large enough so that termite mandibles cannot grasp it, yet small enough so the soft-bodied termites cannot tunnel through it (Ebeling & Pence 1957). Particles must be resilient enough so that they are not crushed under the weight of a structure (Tamashiro et al. 1991). The idea was probably not pursued because of the "golden age" of soil termiticides. However, during the 1980's, while running termiticide trials requiring C. formosanus to tunnel through different substrates, Tamashiro et al. (1991) again observed that tunneling distance was correlated with particle size. In the laboratory and after four years of field studies, Tamashiro et al. (1991) demonstrated that the basaltic barrier was effective in excluding Formosan subterranean termites from structures, if the particles were >1.7 and <2.8 mm. The Basaltic Termite Barrier is refined sandblast sand solid enough to withstand the weight of a structural foundation without crushing. It is currently being marketed in Hawaii as an additional protection against Formosan subterranean termite. The smaller bodied Reticulitermes spp. requires different particle sizes to prevent penetration (Su & Scheffrahn 1992). R. flavipes were more successful in penetrating field tests, but in no case did they completely penetrate the barrier during the 1-3 month trial (Su & Scheffrahn 1992).

Borates are inorganic salts, much like those Wolman investigated in 1898. Two products registered for use on wood in controlling termites are Bora-Care (Nissus, 46% active ingredient (A. I.) in ethylene glycol) and Tim-Bor (U. S. Borax, 90% A. I., powder). The A. I. in both products is disodium octaborate tetrahydrate. These products seemed to cause significant mortality in termites which feed upon treated wood (Su & Scheffrahn 1991a, b; Grace & Yamamoto 1992). In the case of Bora-Care, feeding was significantly reduced on the treated blocks (Grace & Yamamoto 1992). Borates, like insect growth regulators, are perceived as reduced chemical, environmentally friendly pest control tools. However, borates were not shown to be repellent in the soil (Grace 1991a) and should not be used as a soil treatment.

Baits have been experimented with for termite control since at least the early 1900's. Randall & Doody (1934b, p. 475) report the use of "straw or chaff soaked in a solution of sugar and sodium arsenite," as a bait against the harvester termite in the tropics. A bait of 28 g white arsenic or sodium arsenite mixed with 454 g of "treacle" (probably a sugar-based syrup), poured into woodwork, was reportedly used to control termites in Australia (Randall & Doody 1934b). These two undocumented successful uses of termite baits were opposed to the unsuccessful trials of other termite investigators. Randall & Doody (1934b) report that they could not induce drywood, dampwood, or subterranean termites to feed on baits of 10% white arsenic and honey or 0.5% sodium arsenite in a dilute sugar solution and were puzzled that the termites

seemed to avoid their sugar-based bait instead of searching for the sugar (Randall & Doody 1934b).

These baits appear to be better suited for ants than termites. Although ants and termites are both social insects, their behavior is quite different. Today we know that the ideal bait must contain a slow-acting toxicant (Su & Scheffrahn 1989a, Su 1991), so termites have time to spread the toxicant to nestmates. The bait should also be non-repellent (Su & Scheffrahn 1989a, Su 1991), so termites do not avoid feeding on it, as they did in Randall & Doody's (1934b) experiment. Lastly, the bait matrix should probably be preferred over alternative food sources within the foraging area (Esenther & Beal 1974).

Su (1994) has achieved successful control of the native and Formosan subterranean termite colonies in urban southeast Florida, baiting with hexaflumuron, an insect growth regulator. Sixty years have passed since the first attempts to use baits for termite control until now. The bait will be marketed as the Sentricon System (DowElanco, Indianapolis, Indiana) starting 1995 and is the first major change in termite control technology in over sixty years.

Statement of Purpose

Evidence from other termite species suggests that populations and territories differ depending on habitat (Lee & Wood 1971). Urban southeast Florida is dramatically different in habitat than the wooded areas of Gainesville, Florida. Foraging dynamics of Reticulitermes spp. colonies under conditions similar to wooded habitats in Gainesville are largely unknown. Two factors

which probably have contributed to the lack of information on the ecology of subterranean termites are the successful use of termiticides and the absence of tools with which to study these cryptic insects.

With increasing environmental concerns, termiticide soil drenches have rapidly fallen out of favor with the general public. Major urban pests, with the exception of termites and fleas, have some reduced chemical control alternatives. In order to generate a reduced chemical control program, the biology and behavior of the pest must be thoroughly studied. The cryptic and social nature of subterranean termites made ecological studies almost impossible to undertake until the advent of dye markers. Dye markers have revolutionized the way termitologists study the foraging behavior of subterranean termites. Termitologist can follow the territory changes in colonies over a period of years via multiple mark-recapture using a long-term dye marker such as Nile Blue A (Su et al. 1991a) instead of being limited to destructive direct sampling methods such as excavation.

In order to provide more information on the biology and behavior of subterranean termites so that reduced chemical tactics can be more efficiently employed, the purpose of this research program was to evaluate foraging dynamics in wooded areas of Gainesville, Florida, and attempt colony suppression or elimination with the IGR, pyriproxyfen. In order to effectively achieve this objective, more long-term dye markers need to be identified to follow multiple field colonies in a given area. Currently, only Nile Blue A appears

to be a suitable long-term marker for Reticulitermes spp. In addition, the possible effect of food choice on bait acceptability must be considered. The effect of the active ingredient, a juvenile hormone mimic, on colony survival must also be examined in order to implement a monitoring program of reduced chemical control for subterranean termites.

CHAPTER 2
FORAGING TERRITORIES AND ESTIMATES OF FORAGING POPULATION SIZE FOR RETICULITERMES SPP. COLONIES IN WOODED AREAS OF GAINESVILLE, FLORIDA

Introduction

Despite the economic importance of the Reticulitermes spp. (Su & Scheffrahn 1990a), only recently has the foraging dynamics of this important genus been studied (Esenther 1980a, Howard et al. 1982, Grace et al. 1989, Grace 1990, Su et al. 1993). Nutting & Jones (1990) reviewed the techniques for studying the ecology of subterranean termites. These techniques included direct excavation, examining spatial patterns of attack on vegetation, use of bait grids, or the presence of agonisitic interactions. Most of the sampling methods used were destructive, thus eliminating the use of the colony for long-term studies. Another disadvantage of destructive sampling is that populations and territories may be underestimated because the extent of the gallery system and nest location are usually unknown.

Mark-recapture techniques are minimally disruptive sampling methods, but these techniques have many disadvantages concerning the validity of biological and statistical assumptions. Biological assumptions include zero net migration for the population, use of an appropriate marker, obtaining a random sample for marking, and random mixing of marked individuals. The relatively

short interval to complete the weighted means, triple-mark-recapture (≈40 d) suggests that net migration is probably zero. Marking difficulties are discussed in Chapter 3 on Testing Dye Markers for Reticulitermes spp. More serious is the question of obtaining a random sample of the population which would involve theories in foraging behavior. Virtually uninvestigated at this time is the possibility of division of labor within the worker caste and its effect on foraging (Brian 1965, Brian Forschler, personal communication). Factors such as loss or recruitment (i. e. lack of random mixing) within a population affect the validity of mark-recapture statistics, and terms in the formulae can also be subject to sampling variation (Roff 1973a, b).

Despite these critical short-comings, mark-recapture remains one of the better tools for studying long-term foraging patterns. Much of the data on Reticulitermes spp. foraging population sizes and foraging territories have been obtained using the weighted-means triple-mark-recapture technique (Su et al. 1993) or the Lincoln Index (Esenther 1980a, Grace et al. 1989, Grace 1990). The purpose of this study was to characterize Reticulitermes spp. colonies in predominantly wooded environments, in Gainesville, Florida, using the triple mark-recapture method of Su & Scheffrahn (1988b). Information generated from this study will provide the background data on these colonies for assessment of baiting attempts using pyriproxyfen (see Chapters 6 & 7).

Materials and Methods

Field sites. Pine stakes were placed approximately 1 to 2 m apart at four field sites in Gainesville, Florida: Animal Science I (ANSCI I), Animal Science II (ANSCI II), Horticulture Unit (HU), and Austin Cary Memorial Forest (AC). Four field sites were also set up on the property of Disneyworld, Orlando, Florida. Initially, at least a 10 X 10 m area was staked. Termites were allowed to forage and infest stakes. Sites were expanded as stakes were infested around the area perimeter. Infested stakes were replaced by underground monitoring stations (Su & Scheffrahn 1986). Activity of the traps was recorded monthly.

Animal Science I and II are part of a property purchased by the University of Florida Foundation in 1986. Pine (Pinus spp.) and oak (Quercus spp.) were the predominant tree species in the area. During the spring and summer, poison ivy (Toxicodendron radicans (L.) Kuntze) was commonly found covering the ground. Leaf litter was at least 1 to 2 cm thick in most areas. The property previously belonged to the Bjornson family but was not built on for at least 50 years (Bruce Delaney, UF Foundation, Realtor, personal communication). Remnants of a dwelling exist about 200 m from Bivens Arm. The soil type in this area was characterized as the Blichton Series and is described as poorly drained (U. S. Soil Conservation Service 1977). The surface layer is about 2.0 cm of very dark gray sand. The subsurface layer is 10.2 cm of gray sand. The subsoil is gray sandy loam in the upper 1.57 cm; dark gray, sandy, clay loam at 11.8 to 25.6 cm; and gray, clay loam to 30.3 cm. At >30.3 cm, the soil is gray

stratified loamy and sandy material. Slopes range from 0 to 8% (U. S. Soil Conservation Service 1977).

Field site ANSCI I was initiated in September 1990. Three actively infested traps was the minimum number deemed necessary to define foraging territories. Nine months after site initiation, four traps were actively infested, thus the first mark-recapture cycle was started on May 28, 1991. Field site ANSCI II was initiated in October 1990. Eleven months after site initiation, three traps were actively infested, thus the first mark-recapture cycle was started on September 10, 1991.

The Horticulture Unit (HU) located at 7922 NW 71st Street, Gainesville, Florida, 32611. Field site HU was initiated in March 1991 in the wooded area to the south of the unit offices. Pine (Pinus spp.) and oak (Quercus spp.) were the predominant tree species in the area. Leaf litter was at least 2 cm thick in most areas. The soil type was characterized as the Flemington Series which is also poorly drained. These soils have a surface layer of very dark gray, loamy sand and a subsurface layer of gray, loamy fine sand that total 3.5 cm. The subsoil is dark gray and gray clayey to depths of 20.9 cm. Slopes range from 1-12% (U. S. Soil Conservation Service 1977). Six months after site initiation, nine traps were actively infested, thus the first mark-recapture cycle was started on September 3, 1991.

The Austin Cary Memorial Forest is 2,100 acres of flatwood preserve under the management of the University of Florida, Department of Forestry. The

study site was initiated on September 5, 1990. The history of the forest and a map of the memorial are recorded by Newins (1937) and Reinsmith (1937). The study site fronted the memorial lodge and was subject to disturbance by forest visitors. A road circled the perimeter of the study area. The tree species in the area were predominantly slash pine (Pinus elliottii Engelm), longleaf pine (P. palustris Mill.), and some oak (Quercus spp.). The area soil type belongs to the Pomona Series which was characterized as poorly drained soil of the flatwoods and coastal plains (Dan Schultz, Austin Cary Forester, personal communication).

Four field sites within the property of Disneyworld, Orlando, Florida, were established. Three sites were on undeveloped parts of the property. The other site was established around the Canada World Showcase in July 1993. The three undeveloped field sites contained R. flavipes and R. virginicus in fallen logs and branches. Termites would infest stakes but would not re-infest traps, so mark-recapture studies could not be done. These sites were abandoned. The site at the Canada Showcase was actively infested with R. flavipes and R. virginicus. Several buildings on the site were also infested. Stakes were actively infested within one month of placement. Traps were set and a mark-recapture cycle was begun in November 1993.

Foraging population estimation. Up to three cycles of the weighted-means mark-recapture method (Begon 1979) were used to determine the population size of colonies at ANSCI I, ANSCI II, HU, AC and Disneyworld (Appendix A). One centrally located trap was recovered from the field site.

Termites were gently knocked from the wooden trap pieces into a clean metal tray and isolated from debris by allowing them to walk on a wooden ramp (Tamashiro et al. 1973) or by allowing them to cling to sheets of moistened filter paper. Termites were then tapped off into a clean metal tray. Numbers and weights of soldiers, presoldiers, workers, nymphs and alates (if present) were recorded (Appendix B). Numbers of workers were determined gravimetrically by weighing five groups of 10 workers.

Termites from the centrally located trap were then allowed to feed on filter paper (9.0 cm, Whatman 2) stained with 0.10% (wt/wt) Nile Blue A (Su et al. 1991a) for 3 to 5 d. Numbers of stained individuals were recorded before being returned to the field trap from which they were originally taken. (If primary or supplementary reproductives were recovered, they were not returned to the field.) Termites were allowed to forage for 7 to 10 d. All the traps were then collected and processed as described above. Termites from traps with marked individuals were fed on stained filter paper, as described above. The mark-release-recapture cycle was then repeated up to two more cycles.

The percentage of stained individuals from each trap was calculated as a measure of the relative proportion of uniformity to validate the random mixing assumption (Appendix A). To test whether stained termites were distributed randomly ($\sigma^2=\mu$), the variance to mean ratio was used as an index of dispersion ($\chi^2=s^2\,(n-1)/\,\bar{x}$)) at the 95% confidence level, where s^2 was the sample variance, n was the sample size, and $\bar{x}$ was the average number of termites in the

sample. If $\sigma^2<\mu$, then the distribution was classified as uniform (regular); if $\sigma^2>\mu$, then the distribution was considered contagious.

The Poisson series ($\sigma^2=\mu$) was used as a model for the random distribution (Elliott 1971). The following conditions must have been met for agreement with the Poisson series: 1) the probability of a given point in the sampling area being occupied by an individual was small, 2) the number of individuals per sampling unit must have been below the maximum possible number that could occur in the area, 3) the presence of an individual must not have affected the presence of other individuals, and 4) the samples must have been small relative to the population (Elliott 1971). While recruitment in the general population of subterranean termites would seem to violate these assumptions, these assumptions seem reasonably true, if only the stained termites within a foraging population were considered.

Foraging territory. Territories (m^2) and distances (m) were determined for related traps that were deemed to belong to one colony. Traps with stained individuals were considered to belong to one colony. Complications arose when territories were vacated by one colony and invaded by a different colony. Different species obviously belonged to different colonies. The task of species identification was especially difficult between R. flavipes and R. virginicus, if only soldiers and not alates were available. Several diagnostic characters were used in combination with the traditional pronotal character for species separation (Banks 1946). Labral characters were examined (Hostettler et al. 1994), and

assuming the weight of foraging individuals is homogenous, the five groups of 10 workers were weighed from selected traps then subject to a Student's t-test when two traps were in question or subject to Tukey's Studentized range test when more than two traps were considered (SAS Institute 1985). The presence of agonistic interactions between the two termite species also aided in delimiting foraging territories. The use of agonism to delimit foraging areas of sympatric species was thoroughly reviewed by Thorne & Haverty (1991). R. virginicus also tended not to cross the wooden apparatus (Tamashiro et al. 1973) used to separate termites from the debris, if it had been previously used with R. flavipes.

Results

Assumption of random mixing. Stained termites appeared to randomly mix among traps which were connected. Nine of 11 sets of χ^2 values indicated that the distribution of stained termites was random and agreement with the Poisson series was accepted at the 95% confidence level for these nine. The remaining two χ^2 values indicated contagious distributions.

ANSCI I. Stained termites released in trap 1 were retrieved from traps 4, 5, and 10 during the first recapture period (Appendix A, Figure 2-1A). The longest foraging distance at this time was 6 m between traps 1 and 10 (Figure 2-1A). After the second recapture period, no new traps were found to contain stained termites (Figure 2-1B). However, trap 1 which was used to start the mark cycle, did not contain any stained termites. During the third marking period, the possibility of the termites in trap 1 being replaced by another species

was not considered, so termites from all traps, including trap 1, were stained and released into their respective traps. Weights of workers from traps with marked termites subject to Tukey's Studentized range test (α=0.05, df=19) (Figure 2-1D) indicated that termites from traps 5 and 10 belonged to one colony of R. flavipes. This colony was named ANSCI IA (Figure 2-1C). Termites of traps 1, 4, 13, and subsequently, 14 belonged to a colony of R. virginicus. This colony was named ANSCI IB (Figure 2-1C). Trap 1 is included with ANSCI IB because this was the trap which originally indicated that a distinct colony occupied the space once inhabited by termites from ANSCI IA.

The foraging distance for ANSCI 1A could be slightly greater than the recorded distance (Table 2-1) because the next set of stakes were set about 3 to 4 m beyond number 14, although not depicted in Figure 2-1C. Only one marking interval was obtained for ANSCI IB (Appendix A), which made calculating the foraging population size equivalent to using the Lincoln Index. The use of the Lincoln Index versus the weighted-means triple-mark-recapture method can account for the large difference in standard errors between ANSCI IA and ANSCI IB (Table 2-1).

ANSCI II. Stained termites released from trap 1 (Table 2-2) were retrieved from traps 1 and 3 during the first recapture period. Termites from trap 1 were R. flavipes. Traps 4, 6, and 7 were also active during the first recapture period but did not contain stained workers. During the second recapture period, stained foragers dispersed to also include traps 7 and 8, in addition to traps 1

and 3 (Figure 2-2A). Workers, soldiers, pre-soldiers, and nymphs were recovered from all four traps (Appendix B). During the third marking period, differential staining of nymphs was observed. Larger nymphs (3.403 ± 0.025 mg/nymph, n=3 groups of 10 nymphs) did not stain; smaller nymphs (2.653 ± 0.059 mg/nymph, n=3 groups of 10 nymphs) stained as intensely as workers.

During the third recapture period, stained individuals were not recovered from trap 3 (Figure 2-2B). Using the criteria listed above, trap 3 which was once occupied by R. flavipes, was determined to be subsequently occupied by R. virginicus. R. flavipes from trap 1 and R. virginicus from trap 3 fought when contact was forced. Workers and soldiers of trap 1 were significantly heavier than those of trap 3 (t_W=11.2092, df=8, $p<0.0001$; t_S=18.6023, df=8, $p<0.0000$) which also supports the contention that individuals of trap 1 were R. flavipes and those from trap 3 were R. virginicus (Figure 2-2C). The population size of ANSCI II was 165,915 ± 3,650 (SEM 2.2%) with a maximum recorded foraging distance of 8 m.

Both primary reproductives were recovered from trap 1 on September 10, 1991, when it was selected for the initial marking. Primary reproductives were also recovered from trap 4 on September 23, 1991, during the first recapture period (Table 2-3). All castes of the termite in various stages of development were also recovered from both traps. Of the 11,589 individuals recovered from trap 1, workers comprised 89.9% of the total; nymphs, 7.1%; soldiers, 2.6%; and pre-soldiers, 0.4% (Appendix B). Of the 8,675 individuals recovered from trap 4,

workers comprised 95.1% of the total; nymphs, 0.6%; soldiers, 3.8% (Appendix B); and pre-soldiers, 0.5%. The distance between traps 1 and 4 was 3.6 m (Figure 2-2C).

Trap 1 continued to remain active despite the removal of the primary reproductives. On March 12, 1992, six months after the removal of the primary reproductives, six supplementary reproductives and an egg mass were recovered during routine servicing of the site. Blue foragers also were present indicating that the original colony was still active. During the July servicing, blue individuals were still being recovered in trap 12, a more recent addition to the site. Recovery of stained individuals indicated that the colony was still active and that Nile Blue can remain visible under field conditions for over 10 months.

HU. Trap 8 was selected for initial marking. Both primary reproductives were recovered from the trap. Termites were determined to be R. virginicus. Of the 2,999 foragers captured (Appendix A), 2,137 were released (Table 2-2). During the first recapture period, only traps 6 and 8 were found to be connected (Figure 2-3A). During the second and third recapture periods, all activity was lost in trap 8. Stained termites were recovered only from trap 6. The foraging population at HU was determined to be 17,368 ± 503 (SEM 2.9%) with a maximum recorded foraging distance of 4 m (Table 2-1).

Although the foraging activity of the colony characterized at HU was low, the area staked covered approximately 50 m^2. Seventeen trap stations were monitored through the entire course of the mark-recapture period. Percentage

of traps active during September 1991 ranged between 53% and 70%. Of those active traps, three traps (2, 5, 8) contained both primary reproductives and trap 3 contained the primary male reproductive and 5 supplementaries (Table 2-3). In March 1992, six months after the primaries were removed from trap 2, six supplementaries were recovered during routine servicing (Table 2-3). Five supplementaries were apterous, one was brachypterous. Each supplementary was separated into 9 cm Petri dishes where all supplementaries produced egg masses. Distances to the nearest trap where reproductives were recovered was as follows: traps 2 and 3, ≈2 m; traps 3 and 5, ≈8 m; and traps 5 and 8, ≈8 m.

Austin Cary. Mark-recapture was attempted three times at this site. During the first attempt (May 31, 1991), 10,344 termites from trap 26 were stained and 8,837 were released. During recapture, only trap 26 contained stained termites (54 stained, 123 unstained). Upon returning marked termites, the trap was inactive.

The second attempt (AC2) was preceded by collecting all the traps to determine if stained termites were detectable. No traps contained stained termites. On March 17, 1992; 803 stained termites from trap 16 were released (Table 2-2, Appendix A). Again, only trap 16 contained stained individuals for the duration of the mark-release period. The termite population was estimated to be 12,559±1,648 (SEM 13.12%), but territory could not be determined (Table 2-1).

The third attempt (AC3) to characterize the field population was again preceded by collecting all the traps to detect the presence of stained termites. No traps contained stained termites. Trap 16 was selected for staining on July 15, 1992 (Table 2-2). After three cycles of mark-recapture (Appendix A), the R. virginicus populations was determined to be 2,014,049±48,876 (SEM 2.46%) with a foraging territory of about 36 m^2 and a maximum recorded foraging distance of about 26 m (Table 2-1). The foraging territory and distance were probably underestimated because termites of this colony invaded traps that were on the perimeter of the study area.

Disneyworld. The first cycle was started on November 13, 1993; and 3,554 stained R. virginicus were returned to the field. Upon the first recapture, no stained termites were in the traps in the surrounding area, including the original release trap. Stained termites had vacated the area or were eliminated by another colony. This site was terminated because there was not enough time to track colony movement within the confines of this program.

Discussion

This study, combined with the previous five (Esenther 1980a, Howard et al. 1982, Grace et al. 1989, Grace 1990, Su et al. 1993), clearly indicate that foraging population sizes and territories vary widely for Reticulitermes spp. Foraging models have addressed two types of problems: which items an animal should eat and when to leave the patch (Stephens & Krebs 1986). These problems are not mutually exclusive. Developing a model for Reticulitermes

spp. foraging requires the dissection of factors associated with food choice and patch choice which ultimately lead to colony growth or stability. The problem of food choice is not addressed in this study. However, models of nutritional ecology (Slansky 1982, Slansky & Rodriguez 1987) could eventually be incorporated into a general foraging model for subterranean termites. Factors which may affect "patch choice" are minimally addressed in this study by way of habitat characterization, the effect of orphaning on colonies, and colony movement into territories previously occupied by a different colony.

With the exception of AC3, colonies characterized in wooded areas of Gainesville, Florida, tended to be smaller than those characterized previously using analogous mark-recapture methods (Esenther 1980a, Grace et al. 1989, Grace 1990, Su et al. 1993). The smaller estimates in this study fall close to the range of R. flavipes and R. virginicus populations in Desoto National Forest, Mississippi (Howard et al. 1982). However, comparisons between the studies must be viewed with caution because sampling techniques and population estimation methods were not equivalent.

Site AC3 appears to fall within the limits for colonies characterized by Su et al. (1993) in recreational forest lands of Broward County, Florida. By definition, recreational forest lands would receive disturbance from forest visitors, much like the Austin Cary site of AC3. Thus, while the recreational forests in Broward County and Austin Cary Forest are "undeveloped," they are not "undisturbed." The Florida Division of Recreation and Parks classifies

"natural" or "undisturbed" habitats as those resembling habitats when Ponce deLeon arrived in 1513 (Duever et al. 1987). Although there are virtually no areas in Florida fitting this description, sites ANSCI I & II and HU are not recreational and not usually frequented by people. The most disturbance traps in these field sites received were from curious raccoons and invading ants, predominantly the snap-ant, Odontomachus brunneus Patton; Florida carpenter ant, Camponotus abdominalis floridanus (Buckley); and the red imported fire ant, Solenopsis invicta Buren. These ants were frequently found with brood, nesting in the wooden termite traps at rates of 3.8 to 37.5%.

With the exception of Esenther's (1980a) study, the other four studies attempted site characterization. Much of the description was limited to the vegetation in the area, especially tree species probably because trees are the assumed food source for termites. Howard et al. (1982) provided the most detail with regards to site location and habitat characterization, including habitat history for Reticulitermes spp.

Howard et al. (1982) attempted to draw generalizations about the habitats in which subterranean termites are found in order to provide an explanation for the vast differences in foraging population size and territory for different locations. Also included was personal communication with Whitford & Gentry who suggested that estimated densities of Reticulitermes spp. differed according to habitat in South Carolina. Whitford & Gentry found that the most "disturbed" habitat, a recently control-burned pine area, contained 1,300 termites/m^2. An

unburned pine plantation contained 260 termites/ m^2; lowland hardwood, 220 termites/ m^2; and turkey oak woodland, 6 termites/ m^2 (Howard et al. 1982). Unfortunately, neither the method, nor specific parameters tested to determine population difference with different habitats were recorded. Howard et al. (1982) stated that temperature, soil type, moisture, and season were apparently the most important variables dictating whether termites were found in wood or soil, but this statement appears to be derived from observation rather than testing specific parameters.

Grace et al. (1989) and Grace (1990) suggested that the limited introduction of R. flavipes to Canada possibly lead to a high degree of relatedness between colonies, thus "colony complexes" probably were formed, while Reticulitermes spp. were endemic to the southeast and had smaller colonies. Based on the data presented in this chapter, Su et al. (1993) surveyed the populations of R. flavipes in undeveloped lands and residential areas. Su et al. (1993) concluded that there was no correlation between habitat type and the foraging population size of R. flavipes, but failed to test specific parameters associated with the habitat. They assumed that the termite habitats were different because human classification of the habitats based on land use purposes were different.

Although few termite researchers would disagree that temperature, soil type, moisture, season, and genetic relatedness are important factors in population size and territory, none of the five studies listed here included data to

support their hypotheses. Subterranean termites are so named because they dwell in the soil for the most part. Yet, description of the soil type was neglected in all the Reticulitermes spp. studies discussed. Soil can serve to regulate moisture, critical to subterranean termite survival, and provide a conducive environment for undefined beneficial microbes which may affect termite survival. Lee & Wood (1971) produced a comprehensive work on termites in association with the soils in which they dwell, however, no studies on the effect of soil type on foraging population size and territories for subterranean termites were included. Soil characterizations were presented with this study, but too few colonies were examined to draw any correlations between soil type and foraging population sizes and territories.

The correlation between foraging populations and habitat have been alluded to with other subterranean termite species. Lee & Wood (1971) listed termite abundance and habitat type, suggesting that differences do exist between various forest habitats and their abundance (12-4,450/ m^2). Jones et al. (1987) also attempted to define abiotic and biotic characteristics within a habitat which could affect foraging of Heterotermes aureus (Snyder).

In an attempt to identify parameters which may control foraging in Reticulitermes spp., a correlation analysis was done between foraging distance (m) and population size on data from this and previous studies (Grace et al. 1989, Grace 1990, Su et al. 1993) (Figure 2-4). These results indicate that there is a significant positive correlation between foraging distance and population

size ($n=16$, $r=0.5512$, $p<0.0269$). Removing the data from Colony II of Su et al. (1993), increases the fit to $r=0.8657$ ($n=15$, $p<0.0001$). Su et al. (1993) hypothesized Colony II was large but covered a small territory because two dead oak trees provided food and harborage. This simplistic approach seems to suggest that food and harborage are important factors limiting foraging distances. If sufficient food or harborage is present in a given area, termite territories may not need to be large.

These results may explain, in part, why territories are small in forested areas such as ANSCI I & II, HU, and the studies by Howard et al. (1982). These forested areas are more apt to have large fallen trees and branches laying for long periods of time on the forest floor as opposed to urban situations. The U. S. Soil Conservation Service (1977) defines urban land as areas that are more than 70% covered with parking lots, large buildings, streets, and sidewalks, where the natural soil cannot be observed. Soils are graded and filled for urban use which may promote increased drainage, reducing the risk to death by flooding for subterranean termites. Forschler (personal communication) found that Reticulitermes spp. can remain submerged for 1.1 d and survive, thus flooding may not heavily impact colony survival if drainage is within this time period.

Other factors revealed in this study which may impact colony survival, and therefore, foraging, is the rate of replacement of primary reproductives with supplementaries. In this study, primary reproductives at ANSCI II were replaced

by supplementaries within six months. The colony survived at least 10 months after the removal of the primaries, at which time the study was terminated. The colony orphaned at HU did not expand or move to other traps as the ANSCII II colony did. Six months later, the colony occupying traps 6 & 8 appeared to have been replaced by another colony. No marked individuals were present and worker and soldier weights (Appendix B) were significantly different (t_W=-3.3957, p<0.01457, df=8; t_S=-5.1105, p<0.0004, df=6).

Only a few studies have investigated the long-term effect of orphaning on field colonies. For example, Lenz & Runko (1993) found that the biomass of a single female Coptotermes lacteus (Froggatt) from neotenic females grouped in ≤5 per nest increased from 38 mg three months after orphaning to 150 mg, 24 months after orphaning. If nests contained ≥5 females, the average weight of an individual female was 30 to 40 mg. Furthermore, orphaned colonies were characterized by unseasonal nymph production. None of these observations have been made with Reticulitermes spp.

Also observed in this study was the colony movement between R. flavipes and R. virginicus. Movement of colonies into territories previously occupied by another is not well documented for Reticulitermes spp. in a wooded or urban areas. The ability of these termites to inhabit similar niches presents interesting questions concerning foraging dynamics. Anecdotally, these subterranean termites are known to be more mobile than C. formosanus. Movement of C. formosanus into territories of R. flavipes has been documented (Su & Scheffrahn

1988b). In this study, laboratory observations revealed that R. flavipes always defeated R. virginicus in combat. However, in a field situation, R. virginicus seemed to inhabit territories after R. flavipes. Competition in the field between other termite species in overlapping niches has been investigated (Jones & Trosset 1991). However, references to colony movement due to competition between Reticulitermes spp. may be inappropriate at this point. Intraspecific competition would be even more difficult to establish because Reticulitermes spp. are not always combative (Thorne & Haverty 1991).

In summary, colonies investigated in wooded areas in Gainesville, Florida, were smaller than those previously recorded. Multiple colonies present at a given field site could not be studied because Nile Blue A was the only available dye marker for Reticulitermes spp. Additional markers are needed to study multiple colonies simultaneously. More dyes for staining Reticulitermes spp. are investigated in Chapter 3.

Table 2-1. Population size calculated after the mark-recapture, maximum foraging distance measured, and the mean worker and soldier weights for all Reticulitermes spp. colonies characterized in Gainesville, Florida

Colony	Population Size (±SEM)	Foraging distance (m)	Mean Worker Weight (±SEM)	Mean Soldier Weight (±SEM)
ANSCI IA[a]	95,821±2,202	6	2.35±0.17	4.45±0.12
ANSCI IB[b]	407,524±58,217	8	2.09±0.07	3.04±0.16
ANSCI II[a]	165,915±3677	8	2.48±0.17	3.76±0.27
HU[b]	17,368±500	4	2.04±0.13	3.19±0.04
AC2[b]	12,559±1,648	0	1.32±0.02	1.93±.
AC3[b]	2,014,049±48,876	26	1.82±0.09	2.49±0.08

[a] R. flavipes
[b] R. virginicus

Table 2-2. Total number of termites captured (n_i), the number of marked termites of the total number captured (m_i), and the number of marked termites released (r_i) during the mark-recapture for time (i) for colonies of Reticulitermes spp. in Gainesville, Florida

Colony		Mark Recapture Interval (i)			
		1	2	3	4
ANSCI IA[a]	Total Captured (n_i)	---	9,470	4,101	7,452
	Mark Captured (m_i)	---	484	815	616
	Marked Released (r_i)	3,396	8,171	3,684	---
ANSCI IB[b]	Total Captured (n_i)	---	13,357	---	---
	Mark Captured (m_i)	---	48	---	---
	Marked Released (r_i)	1,495	---	---	---
ANSCI II[a]	Total Captured (ni)	---	6,673	15,783	6,988
	Mark Captured (m_i)	---	52	484	1,500
	Marked Released (r_i)	5,307	5,943	11,635	---
HU[b]	Total Captured (n_i)	---	6,433	1,130	1,180
	Mark Captured (m_i)	---	422	259	526
	Marked Released (r_i)	2,137	5,252	985	---
AC2[b]	Total Captured (n_i)	---	541	258	---
	Mark Captured (m_i)	---	25	34	---
	Marked Released (r_i)	803	459	---	---
AC3[b]	Total Captured (n_i)	---	45,632	13,325	25,597
	Mark Captured (m_i)	---	565	373	761
	Marked Released (r_i)	16,914	43,980	12,247	---

[a] R. flavipes
[b] R. virginicus

Table 2-3. Dates, location of collection, and weights for primary and supplementary reproductives for colonies of Reticulitermes spp. in Gainesville, Florida

Date	Colony	Trap	Queen	King	Supplementaries
9/10/91	ANSCI II[a]	1	No data	No data	
9/23/91	ANSCI II[a]	4	18.1	46.0	
3/12/92	ANSCI II[a]	1			6 collected, no weight data
9/3/91	HU[b]	8	No data	No data	
9/16/91	HU[b]	2	19.5	5.1	
		3			11.1, 12.6, 12.8, 10.9, 10.2
		5	17.0	4.2	
3/12/92		2			12.4, 9.1, 7.8, 6.2, 7.8, 5.4

[a] R. flavipes
[b] R. virginicus

Figure 2-1. Changing R. flavipes and R. virginicus territories in field site ANSCI I during the first recapture (A), second recapture (B), third recapture (C). Tukey's Studentized range test to separate colonies into ANSCI IA and IB by worker weight.

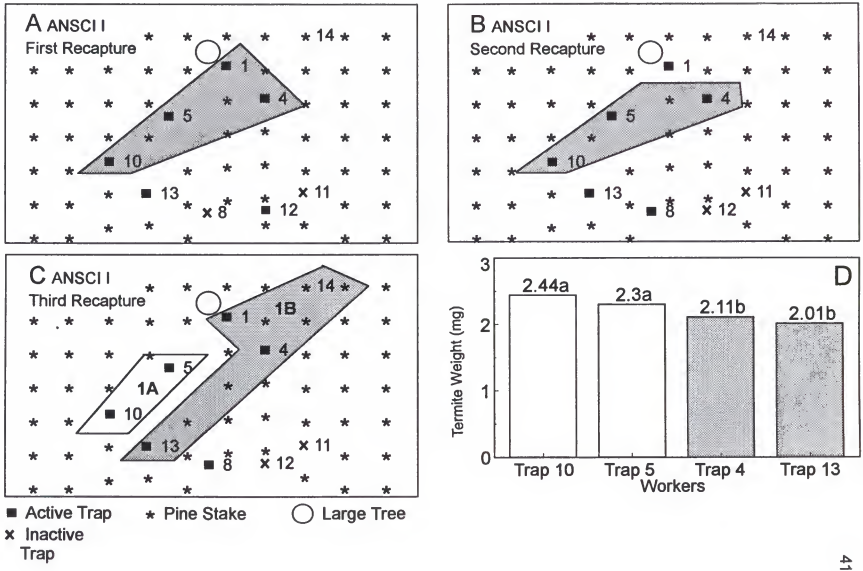
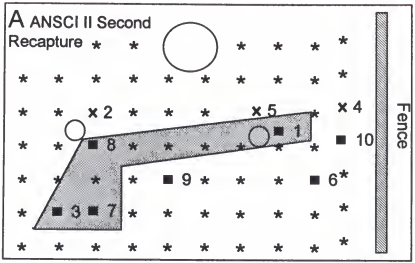
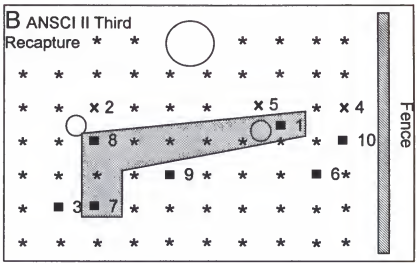

A ANSCI I
First Recapture
B ANSCI I
Second Recapture
C ANSCI I
Third Recapture
1A
1B
D
2.44a
2.3a
2.11b
2.01b
Termite Weight (mg)
Trap 10
Trap 5
Trap 4
Trap 13
Workers
Active Trap
Pine Stake
Large Tree
Inactive Trap

Figure 2-2. Changing R. flavipes territories in field site ANSCI II during the second (A) and third to demonstrate the presence of separate colonies (C).


A ANSCI II Second Recapture
Fence
■ Active Trap
× Inactive Trap4
*Pine Stake
Large Tree


B ANSCI II Third Recapture
Fence

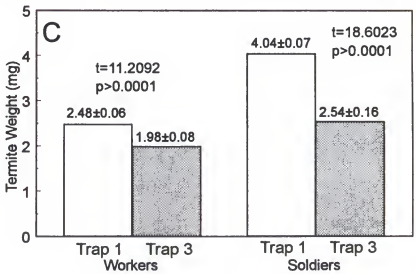
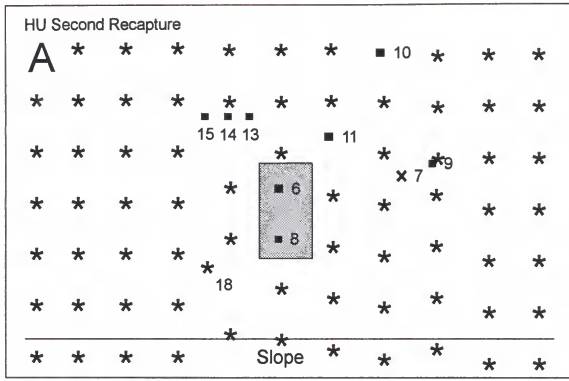
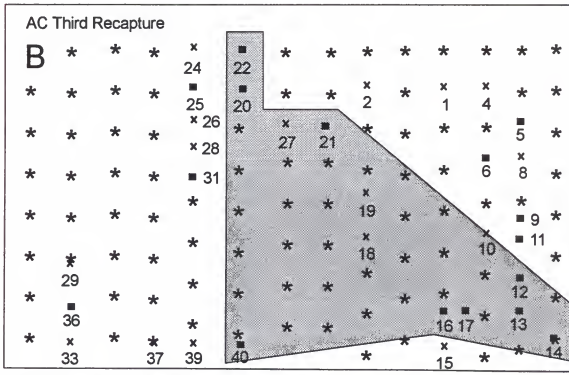

C
Termite Weight (mg)
t=11.2092
p>0.0001
2.48±0.06
1.98±0.08
4.04±0.07
t=18.6023
p>0.0001
2.54±0.16
Trap 1
Trap 3
Workers
Trap 1
Trap 3
Soldiers

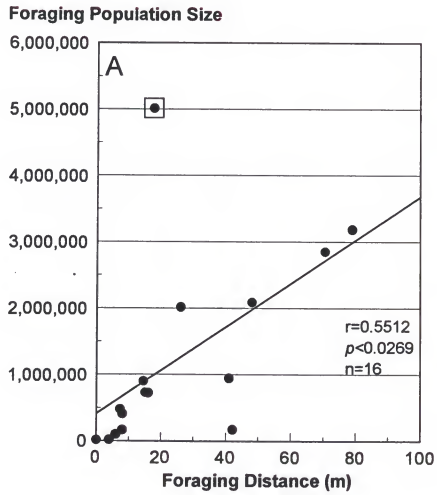
Figure 2-3. Maximum territory of R. virginicus at HU which was during the second recapture period (A). Maximum territory of R. virginicus at AC during the third recapture period (B).

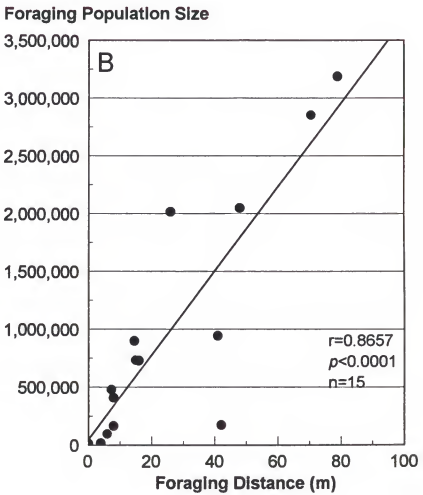




▪ Active Trap ✱Pine Stake
× Inactive Trap

Figure 2-4. Correlation of foraging population size and foraging distance of Reticulitermes spp. with (A) and without (B) data from Colony II (Su et al. 1993).


Foraging Population Size
6,000,000
5,000,000
4,000,000
3,000,000
2,000,000
1,000,000
0
A
r=0.5512
p<0.0269
n=16
0
20
40
60
80
100
Foraging Distance (m)


Foraging Population Size
3,500,000
3,000,000
2,500,000
2,000,000
1,500,000
1,000,000
500,000
0
B
r=0.8657
p<0.0001
n=15
0
20
40
60
80
100
Foraging Distance (m)

CHAPTER 3
STAINS TESTED FOR MARKING R. FLAVIPES AND R. VIRGINICUS (ISOPTERA: RHINOTERMITIDAE)

Introduction

In 1986, over $1 billion was expended by consumers to prevent or control termite infestations, or to repair damage caused by termites (Su & Scheffrahn 1990a). Largely due to the overwhelming success of termiticides applied as a soil drench, research on the biology and foraging behavior of the subterranean termites of Reticulitermes spp., has been neglected. The resulting paucity of data on termite biology and behavior has delayed the implementation of alternative control measures such as baits (Esenther & Beal 1974, Su 1993a, b, 1994) and physical barriers (Ebeling & Pence 1957, Tamashiro et al. 1991, Su & Scheffrahn 1992).

Nutting & Jones (1990) listed several techniques for studying the ecology of subterranean termites which include baiting, quadrat sampling, and exhaustive trapping. These sampling methods can be destructive, thus eliminating the use of a colony for long-term studies. Within the last two decades, researchers have begun using mark-recapture methods for estimating foraging populations and foraging territories of subterranean termites (Lai 1977, Lai et al. 1983, Su & Scheffrahn 1988b, Grace et al. 1989, Grace 1990). Mark-

recapture methods have the benefit of being minimally disruptive, so field sites may be used for continuous studies of colony demographics.

The predominant mark-recapture method used has been the Lincoln Index (Lai 1977, Esenther 1980a, Grace et al. 1989, Grace 1990). The Lincoln Index utilizes only one marking interval and can lead to large standard errors in foraging population estimation. Using the Lincoln Index, Esenther (1980a) reported R. flavipes population figures of 1,135,000 ± 736,400 (65% SEM), 325,600 ± 152,100 (47% SEM), and 9,516,300 ± 4,255,800 (45% SEM), while Grace (1990) reported figures of 3,187,538 ± 606,341 (19% SEM) and 2,084,219 ± 323,049 (15% SEM). The weighted-means, triple-mark-recapture technique (Begon 1979) has been shown to consistently produce lower standard errors (Su & Scheffrahn 1988b, Su et al. 1993, see Chapter 2).

Studies of the possible interactions of multiple colonies within a given site, using mark-recapture methods, have been limited by the number of dye markers available (see Chapter 2). Stains must not cause significant immediate or delayed mortality; stains must be retained by the termites for the desired mark-recapture cycle; and termites must not illicit any behavioral changes due to unidentified direct or indirect consequences of the staining process which might make them unacceptable to colonymates or affect foraging behavior. If the stain is to be used for foraging population estimation, the dye cannot be passed among colonymates by trophallaxis.

Numerous biological stains have been investigated as suitable markers for population estimation of subterranean termites (Lai 1977, Lai et al. 1983, Grace & Abdallay 1989, 1990; Esenther 1980a, Su et al. 1983, Su et al. 1988). Due to the stringent conditions listed above, only Sudan Red 7B and Nile Blue A (Lai et al. 1983, Su et al. 1983) have been used for marking C. formosanus. Neutral Red (Esenther 1980a), Sudan Red 7B (Grace et al. 1989, Grace 1990), and Nile Blue A (Su et al. 1993) have been used for marking R. flavipes. Neutral Red and Sudan Red 7B have been used for short-term foraging studies using the Lincoln Index (Esenther 1980a, Grace et al. 1989, Grace 1990). Sudan Red 7B is probably not suitable for the long-term, triple-mark-recapture method with R. flavipes; it has been reported to cause high delayed mortality and is not retained for more than 15 days in this species (Su et al. 1988).

Su et al. (1991a) have determined that Nile Blue A is suitable for marking R. flavipes. However, the dye persists in R. flavipes for up to ten months in the field (see Chapter 2). Thus, mark-recapture studies that utilize Nile Blue A can only be used on one colony in a study area every year without confounding results.

The primary objective of this study was to identify suitable dyes and concentrations for a multiple marking system for a long-term mark-recapture method. A secondary objective was to identify stains which did not cause high mortality, but lacked the persistence to be useful in long-term field studies. Stains fitting criteria for the secondary objective could be useful in short-term

field or laboratory studies. We attempted to identify colors other than blue and red because Nile Blue A and Neutral Red are already proven to be suitable stains for marking Reticulitermes spp. Most of the stains used by Su et al. (1991a) and Salih & Logan (1990) were determined to be acid (anionic), often with sulfur substituents (Conn 1953, Green 1990). Acid dyes generally cannot penetrate healthy, living cells (Ivanov 1987). In the studies by Su et al. (1991a) and Salih & Logan (1990), stains of the xanthene class were always fatal (Conn 1953). Thus, the stains selected for this study were predominantly basic (cationic) stains, generally containing nitrogen and oxygen in lieu of sulfur groups, and belong to stain classes whose chemical structure resemble that of Nile Blue A, Neutral Red, or Sudan Red 7B (Conn 1953, Green 1990).

Materials and Methods

For each replicate, two Petri dishes (9.0 cm diam., 1.5 cm high) were prepared with the following treatments applied to filter paper (9.0 cm, Whatman 2): distilled water control, solvent control, and dyes dissolved in their respective solvents at concentrations of 0.05, 0.10, 0.25, 0.50, 1.00, and 2.00% (wt/vol). In some cases, concentrations were decreased because the filter paper was strongly stained at the lower concentrations.

R. flavipes or R. virginicus were used within two weeks of being field collected. About 0.5-1.0 ml of termites per Petri dish were allowed to feed on stained filter paper for 3 to 5 d. If stains did not cause significant mortality at the end of the 3 to 5 d feeding period, then 20 stained workers were weighed in

groups of 10, introduced into a new Petri dish (5.5 cm), and allowed to feed on untreated filter paper moistened with distilled water. Number of dead and stained termites were scored daily for 15 d. More than one termite colony was used when available.

Each Petri dish was considered one experimental unit in a completely randomized design. For each stain, termite survival and body weight at 15 d were analyzed by analysis of variance. Concentration was the class variable. Significant difference among means ($p<0.05$) were separated by Tukey's Studentized range test (SAS Institute 1985).

Results and Discussion

Acid Blue 29 and Acid Red 114, anionic disazo dyes; and Acid Violet 5, an anionic monoazo dye, did not cause significant mortality for concentrations ≤ 0.50% (Table 3-1). Acid Blue 29, Acid Red 114, and Acid Violet 5 were limited to the gutline of the termite, and unlike Nile Blue A and Neutral Red, did not stain tissue in the abdomen or the head capsule. These stains were reliably detected for about 2 d. Thereafter, staining was faintly detected up to 4 and 5 d, but stained termites were often difficult to distinguish from the controls. By 15 d, 0% of the termites were stained (Table 3-1). Acid Blue 29, Acid Red 114, and Acid Violet 5 were quickly eliminated in the feces and were probably passed by coprophagy or trophallaxis. There is no evidence in the literature that Acid Blue 29, Acid Red 114, and Acid Violet 5 have been used in biological applications; however, industrial uses include textile applications such as dyeing wool and

silk, as well as non-textile applications such as coloring inks and soaps (Green 1990).

Brilliant Cresyl Blue ALD and 8-Dimethylamino-2,3-benzophenoxazine (DIMETH) are oxazines, as is Nile Blue A. Brilliant Cresyl Blue, ≤0.25%; and DIMETH, ≤0.50%, did not cause significant mortality (Table 3-2). Termites were stained faintly in the head capsule and throughout the abdomen, not just along the gutline. Stains persisted for the 15 d duration or until the test was prematurely terminated due to termite mortality at the higher concentrations. The percentage of termites which retained the stain for 15 d at the lower concentrations (0.025-0.25%) without significant mortality were faint and not useful for field studies (Table 3-2). Termites stained at higher concentrations were visibly blue, but mortality was significantly greater than the controls (Table 3-2). Brilliant Cresyl Blue is used almost exclusively as a biological stain with numerous applications (Green 1990).

Brilliant Green, Malachite Green, Methyl Green, and Pararosaniline are cationic triphenylmethane dyes. Workers were stained only along the gutline. Stained workers could be reliably detected for about 2 d (Table 3-3). There after, coloring was too faint to be useful in field applications, although staining was present for several more days. By 15 d, 0% of the termites were stained, except at 0.05% Brilliant Green (Table 3-3). Brilliant Green, Malachite Green, Methyl Green, and Pararosanile have numerous biological and industrial

applications, including the indicator properties of Brilliant Green with a visual transition interval of pH 0.0 (yellow) to pH 2.0 (green) (Green 1990).

Crystal Violet is a triarylmethane dye and is closely related to the triphenylmethane dyes. Termites which fed on this stain were noticeably less vigorous than control termites. Although there was no significant difference in survivorship as concentrations increased, there was a definite trend of increasing mortality with increasing concentration (Table 3-4). Termites which fed on filter paper stained at 1.00 and 2.00% Crystal Violet were killed before they could be transferred to untreated filter paper. Worker weight also tended to decrease with increasing stain concentration, but there were no significant differences in weight loss (Table 3-4). The stain was reliably visible along the gutline for about 2 d, then became increasingly difficult to distinguish from control termites. By 15 d, 0% of the termites were stained and termites did not regain their vigor (Table 3-4). Crystal Violet is used extensively as a Gram-positive stain and as a spirochete stain, as well as industrially (Green 1990).

Direct Orange and Direct Red are anionic disazo dyes. There was no significant difference in mortality or worker weight with increasing concentrations of Direct Orange (Table 3-5). However, mortality and mean weight were significantly different than controls for 1.00 and 2.00% Direct Red (Table 3-5). Stains were reliably detected along the gutline for 2 to 3 d and were increasingly difficult to detect thereafter. By 15 d, only 31% of the termites were stained at 2.00% Direct Orange, and 0% were stained at all concentrations of Direct Red

(Table 3-5). Direct Orange and Direct Red have been used on cotton, wool, and silk. Direct Orange has not been used as a biological stain; Direct Red has been used, biologically, as a contrast stain (Green 1990).

Fast Green FCF is an anionic triphenylmethane and appeared to have longer persistence than stains in the cationic triphenylmethane class. There was no significant mortality or mean weight loss with increasing concentration, even at 1.00 and 2.00% Fast Green (Table 3-6). Percentage of termites stained at 15 d was only 58.2% (Table 3-6). Termites stained a bright green along the gutline. The stain was rapidly excreted, even re-staining the untreated filter paper. Under these conditions, Fast Green was reliably detected for about 7 d before becoming faint. However, workers may have fed on the feces-stained filter paper or passed Fast Green trophallactically despite efforts to replace the feces-stained filter paper. Under field conditions, Fast Green may last <7 d. Fast Green may be useful in behavioral laboratory studies investigating agonistic interactions or studies where feeding of individual termites needs confirmation because of its readily visible color. Fast Green is used in numerous biological staining procedures, such as plant histology and cytology (Green 1990). It was previously a general food dye, but is now a suspected carcinogen.

Janus Green B, Neutral Red, and Safranin O are cationic azine dyes. There was no significant difference in mortality at concentrations ≤0.25% Janus Green; and ≤0.50% Neutral Red and Safranin O (Table 3-7). Mortality was significantly greater than controls at the higher concentrations (Table 3-7).

There was no significant difference in mean weight with increasing concentration for Janus Green, but mean weight decreased with increasing concentration for Neutral Red and Safranin O (Table 3-7). Termites which fed on Neutral Red and Safranin O were colored in the head capsule and through the abdomen, not just along the gutline. Termites which fed on Janus Green were mauve-colored and not useful for field applications because the natural food source of termites could leave a similar color. Termites which fed on Neutral Red and Safranin O began to lose color intensity at different rates. Although Neutral Red lasted for 15 d in 100% of the termites at 0.10-0.50% (Table 3-7), the more faintly stained termites would be difficult to distinguish from termites which fed on reddish material in the field. Termites stained with Neutral Red would probably not retain coloration long enough to complete the triple-mark-recapture method (≈40 d). Termites stained with Safranin O appeared to be less vigorous than controls. Janus Green is an important biological stain for mitochodria and is also used with Neutral Red to stain living blood cells (Green 1990). Neutral Red has numerous biological applications and has limited use as a pH indicator (pH 6.8, red; pH 8.0, yellow) (Green 1990). Safranin O also has numerous industrial and biological applications (Green 1990).

Methylene Blue and Toluidine Blue are cationic thiazine dyes. Mortality increased with increasing concentration and became significantly different than controls at the higher concentrations (Table 3-8). Mean worker weight significantly decreased with increasing concentrations of the stains (Table 3-8).

Stains were faint but not limited to the gutline, thus probably not useful for field applications. Termites which fed on >0.50% Methylene Blue and Toluidine Blue became dorsoventrally flattened, indicative of impaired feeding and water regulation. At 15 d, 100% of the termites were stained at 0.05-0.25% Toluidine Blue, however, mortality at 0.10 and 0.25% were significantly higher than controls (Table 3-8). Methylene Blue and Toluidine Blue have numerous biological applications (Green 1990).

Oil Red O is a neutral disazo dye. There was no significant difference in mortality for concentrations ≤0.25%, although mortality increased with increasing concentration (Table 3-9). Mortality at 0.50 and 1.00% was significantly greater than that of the controls. There was no significant difference in termite weights, although weight tended to decrease with increasing stain concentration. Staining was not limited to the gutline. Termites were stained through the abdomen and in the head capsule. By 15 d, only 50% of the termites were stained at 0.10% Oil Red, the highest concentration where termite mortality was not significantly different than controls (Table 3-9). Oil Red O is used to color oils and cosmetics, and is used to demonstrate lipid degeneration in the central nervous system (Green 1990).

Little information exists on Reactive Green. There was no significant difference in mortality (Table 3-10). Only termites which fed on 2.00% Reactive Green were significantly lower in weight than the controls. There was no significant difference in all other concentrations. Termites were stained only

along the gutline. Visible coloration could be detected for about 2 d, but the stain persisted in the termite for longer periods (Table 3-10). By 15 d, 0% of the termites were stained at all concentrations, except 0.50% (Table 3-10).

Sudan IV is a disazo dye. There was no significant mortality for concentrations ≤0.50% Sudan IV (Table 3-11). Between 93.4 and 100% of the termites retained Sudan IV for the 15 d interval for all concentrations of stain (Table 3-11). With increased sample size, perhaps mortality at 1.00 and 2.00% would have been significantly different than the distilled water and acetone controls. At 1.00 and 2.00%, Sudan IV was crusted onto the filter paper and water beaded on the top of the filter paper disc instead of being absorbed. There was no significant difference in weight for all concentrations of Sudan IV. Su et al. (1991a) found Sudan IV to be a good dye for R. flavipes at concentrations of 0.05, 0.25, and 0.50%. In this study, R. virginicus, a smaller termite, was observed to be less vigorous when feeding on Sudan IV, which may have resulted in delayed mortality or non-acceptance by colonymates. However, Sudan IV is a red stain and no further testing was done with this stain because Neutral Red is already proven to be a suitable marker.

Sudan Orange is a monoazo dye. There were significant differences in mortality for Sudan Orange concentrations of ≥0.25% (Table 3-11). Weight also significantly decreased with increasing concentration. The stain was visible in the termite gutline for about 2 d. By 15 d, 0% of the termites were stained (Table 3-11). The natural sallow color of the termites made this color a poor choice.

Victoria Blue R is a cationic diphenylnaphthylmethane dye. This stain caused almost complete mortality of termites feeding on treated filter paper in concentrations >0.25% (Table 3-12). Termites which fed on 0.05 and 0.10% Victoria Blue R did not appear colored, thus were not included in Table 3-12. There was no significant difference in mortality, but there was a significant difference in weight of termites at 0.25% when compared with the controls (Table 3-12). By 15 d, 0% of the termites were stained (Table 3-12). Victoria Blue R is not suitable for staining termites for laboratory or field studies.

Under these conditions, none of the stains tested here meet the longevity requirements for a marker to be used with the triple-mark-recapture method (Su & Scheffrahn 1988b). Nile Blue A still appears to be the best long-term marker available. Neutral Red is adequate for single-mark-recapture studies such as the Lincoln Index. Both stains are known to dye lipids (Green 1990). In subterranean termites, Nile Blue A and Neutral Red probably stain the fat body in the abdomen and the head capsule. If the head capsule is stained, even if termites feed on material which obscures the color of the abdomen, marked termites can still be separated from unmarked individuals. Unfortunately, the most available stains through Sigma, Aldrich or Fisher are in the class triphenylmethane which colors only the gutline and is quickly purged.

In summary, twenty-three stains, at several concentrations, were tested for mortality, effect on worker weight, and stain persistence in R. virginicus and R. flavipes. None of the stains investigated met the criteria for a long-term

marker for the triple-mark-recapture method to study colony demographics. However, Neutral Red is adequate for single mark-recapture efforts such as the Lincoln Index. Acid Blue, Acid Red, Acid Violet, Direct Orange, Direct Red, and especially Fast Green FCF may be used for short term laboratory studies of behavior or feeding. Crystal Violet and Victoria Blue R performed poorly, causing high mortality during staining. Sudan IV at concentrations of 0.10 and 0.25% may be suitable, but R. virginicus which fed on Sudan IV at 0.10 and 0.25%, appeared to be less vigorous than controls. Under our conditions, all other stains lacked the color intensity to be useful for the laboratory or field.

Table 3-1 The mean survival (± SEM), percentage stained at test termination, days of stain persistence, and the mean preweight (±SEM) of Reticulitermes spp. workers after staining with the azo stains: Acid Blue 29, Acid Red 114, and Acid Violet 5, at all concentrations tested

Stain	Other Names	Conc. (wt/vol)	Mean Survival[1] (±SEM)	N	%Stained	Persis. (d)	Mean Prewt. (mg/worker)
Acid Blue 29	—	H20	20.00±0.00a	4	0	0	2.13±0.07a
C. I. 20460		EtOH	19.75±0.50a	4	0	0	2.11±0.07a
(Anionic		0.05	19.75±0.50a	4	0	1-2	2.10±0.05a
Disazo)		0.10	19.50±0.57a	4	0	2	2.07±0.06ab
		0.25	18.50±1.73a	4	0	2	1.97±0.09c
		0.50	17.66±0.57a	3	0	2	1.99±0.07bc
		1.00	3.00±4.24b	1	0	2-3	1.94±0.04c
		2.00	1.25±2.50b	2	0	3-5	1.62±0.07d
Acid Red 114	—	H2O	19.75±0.50a	4	0	0	2.16±0.03a
C. I. 23635		0.05	20.00±0.00a	4	0	2-4	2.18±0.04a
(Anionic		0.10	20.00±0.00a	4	0	3-4	2.17±0.06a
Disazo)		0.25	19.75±0.50a	4	0	4-5	2.14±0.07ab

Table 3-1. Cont.

Stain	Other Names	Conc. (wt/vol)	Mean Survival (±SEM)	N	%Stained	Persis. (d)	Mean Prewt. (mg/worker)
		0.50	19.25±0.95ab	4	0	4-5	2.14±0.05ab
		1.00	20.00±0.00a	4	0	4-5	2.08±0.08b
		2.00	16.00±3.65b	4	0	4-5	1.81±0.05c
Acid Violet 5	Lanafuchsin	H20	18.00±2.60a	6	0	0	2.03±0.18a
C. I. 18125	6B	EtOH	19.50±0.83a	4	0	0	2.02±0.18a
(Anionic		0.05	19.25±0.50a	6	0	2	2.13±0.05a
Monoazo)		0.10	17.25±4.19a	6	0	2	2.16±0.07a
		0.25	18.50±1.22a	6	0	2	2.00±0.18a
		0.50	18.00±1.26a	4	0	2	2.00±0.25a
		1.00	4.75±5.85b	4	0	2	1.98±0.08a
		2.00	0.00±0.00b	4	0	2	1.77±0.07b

[1] Mean survival out of a total of 20 termites

Table 3-2. The mean survival (± SEM), percentage stained at test termination, days of stain persistence, and the mean preweight (± SEM) of Reticulitermes spp. workers after staining with the oxazine stains: Brilliant Cresyl Blue ALD and 8-Dimethylamino-2,3-Benzophenoxazine, at all concentrations tested

Stain	Other Names	Conc. (wt/vol)	Mean Survival[1] (± SEM)	N	%Stained	Persis. (d)	Mean Prewt. (mg/worker)
Brilliant	---	H20	18.00±1.41a	2	0	0	2.53±0.13ab
Cresyl Blue		EtOH	19.00±1.41a	2	0	0	2.82±0.20a
ALD		0.0125	19.00±0.00a	2	23.7	11-15	2.56±0.07ab
C. I. --		0.025	19.00±0.00a	2	84.2	15	2.53±0.16ab
(Cationic		0.05	17.50±0.70a	2	94.1	15	2.37±0.10bc
Oxazine)		0.10	19.00±0.00a	2	100.0	15	2.45±0.16bc
		0.25	15.00±0.41ab	2	100.0	15	2.33±0.21bcd
		0.50	7.50±3.53b	2	50.0	11-15	2.17±0.07cde
		1.00	15.50±0.70ab	2	100.0	15	1.97±0.17de
		2.00	7.50±6.36b	2	100.0	15	2.04±0.16e
8-Dimethyl-	Meldola's	H20	17.14±2.47a	7	0	0	2.01±0.55a
amino-2,3-	Blue	EtOH	18.33±1.63a	6	0	0	1.93±0.43ab

Table 3-2. Cont.

Stain	Other Names	Conc. (wt/vol)	Mean Survival (± SEM)	N	%Stained	Persis. (d)	Mean Prewt. (mg/worker)
Benzophen-		0.05	17.71±3.45a	7	63.1	14-15	1.79±0.48abcd
oxazine		0.10	17.00±1.77a	8	96.7	15	1.84±0.48abc
C. I. 51175		0.25	14.90±3.78a	10	88.5	15	1.75±0.55abcd
(Oxazine)		0.50	15.50±2.38a	4	100.0	15	1.41±0.37d
		1.00	0.00±0.00b	8	0	3-9	1.52±0.35bcd
		2.00	0.00±0.00b	6	0	4-5	1.47±0.24cd

[1] Mean survival out of a total of 20 termites

Table 3-3. The mean survival (± SEM), percentage stained at test termination, days of stain persistence, and the mean preweight (± SEM) of Reticulitermes spp. workers after staining with the cationic triphenylmethane stains: Brilliant Green, Malachite Green, Methyl Green, and Pararosaniline, at all concentrations tested

Stains	Other Names	Conc. (wt.vol)	Mean Survival[1] (± SEM)	N	%Stained	Persis. (d)	Mean Prewt. (mg/worker)
Brilliant	Basic	H2O	20.00±0.00a	4	0	1	2.51±0.35a
Green	Green 1	0.05	19.25±0.95a	4	25.0	1-15	2.35±0.34ab
C. I. 42040		0.10	13.25±4.42ab	4	0	2-7	2.10±0.25bc
		0.25	7.50±7.93ab	4	0	2-4	2.00±0.28c
		0.50	11.25±3.86b	4	0	2-8	1.86±0.16c
Malachite	Solvent	H2O	19.50±0.70ab	2	0	0	2.80±0.09a
Green	Green 1	Acetone	20.00±0.00a	2	0	0	2.83±0.06a
C. I. 42000		0.25	16.50±0.70ab	2	0	3-7	2.18±0.07b
		0.50	17.50±3.53ab	2	0	3	2.24±0.11b
		1.00	17.50±0.70ab	2	0	2-3	2.21±0.08b
		2.00	11.50±3.53b	2	0	3	2.09±0.11b

Table 3-3. Cont.

Stains	Other Names	Conc. (wt.vol)	Mean Survival (± SEM)	N	%Stained	Persis. (d)	Mean Prewt. (mg/worker)
Methyl Green	---	H2O	19.50±0.57a	4	0	0	2.31±0.23[3]
C. I. 42590		0.05	19.75±0.50a	4	0	4-8	2.21±0.30
		0.10	18.50±0.57a	4	0	4-14	2.27±0.31
		0.25	15.25±3.86ab	4	0	4-14	2.31±0.14
		0.50	9.50±5.32b	4	0	4-14	2.17±0.13
Pararos-	Basic Red 9	H2O	19.50±1.41[2]	2	0	0	2.79±0.13[4]
aniline		Acetone	19.00±0.70	2	0	0	2.72±0.12
C. I. 42500		0.25	18.50±0.70	2	0	2-4	2.78±0.10

[1]Mean survival out of a total of 20 termites
[2]Pararosaniline, ANOVA on Mean Survival, ns, (df=5, F=0.50, p<0.6495)
[3]Methyl Green, ANOVA on Mean Preweight, ns, (df=59, F=0.84, p<0.5086)
[4]Pararosaniline, ANOVA on Mean Preweight, ns, (df=17, F=0.72, p<0.5049)

Table 3-4 The mean survival (± SEM), percentage stained at test termination, days of stain persistence, and the mean preweight (± SEM) of Reticulitermes spp. workers after staining with Crystal Violet, a cationic triarylmethane stain

Stains	Other Names	Conc. (wt.vol)	Mean Survival[1] (± SEM)	N	%Stained	Persis. (d)	Mean Prewt. (mg/worker)
Crystal Violet	Basic	H20	17.50±2.38a	4	0	0	2.18±0.57[1]
C. I. 42555	Violet 3,	EtOH	17.50±2.12a	2	0	0	1.75±0.12
	Gentian	0.05	18.50±1.29a	4	0	2	2.04±0.61
	violet	0.10	12.00±7.11a	4	0	3-7	1.82±0.49
		0.25	8.00±9.27a	4	0	2-5	1.69±0.43
		0.50	5.25±6.18a	4	0	4-11	1.67±0.43
		1.00	.	.	.	.	.
		2.00	.	.	.	.	.

[1]Mean survival out of a total of 20 termites
[2]Crystal Violet, ANOVA on Mean Preweight, ns, (df=65, F=2.00, p<0.0922)

Table 3-5. The mean survival (± SEM), percentage stained at test termination, days of stain persistence, and the mean preweight (± SEM) of Reticulitermes spp. workers after staining with the anionic disazo stains: Direct Orange and Direct Red, at all concentrations tested

Stains	Other Names	Conc. (wt.vol)	Mean Survival[1] (± SEM)	N	%Stained	Persis. (d)	Mean Prewt. (mg/worker)
Direct Orange	---	H20	17.50±2.44[2]	8	0	0	2.20±0.40[3]
C. I. 23655		0.05	17.87±1.80	8	17.0	1	2.24±0.47
		0.10	17.28±3.49	7	3.9	2-6	2.27±0.48
		0.25	19.62±0.74	8	24.3	5-8	2.28±0.43
		0.50	18.25±1.98	8	22.0	6-9	2.26±0.40
		1.00	17.00±3.96	8	22.9	6-11	2.31±0.47
		2.00	18.75±0.88	8	31.0	6-8	2.18±0.47
Direct Red	Sirius Red	H20	19.25±0.50a	4	0	0	2.10±0.04a
C. I. 28160	4B	0.05	19.50±1.00a	4	0	2-3	2.10±0.08a
		0.10	19.75±0.50a	4	0	2-4	2.16±0.05a
		0.25	19.25±1.50a	4	0	2-4	2.12±0.07a
		0.50	19.00±0.81a	4	0	4-5	2.14±0.04a

Table 3-5. Cont.

Stains	Other Names	Conc. (wt.vol)	Mean Survival (± SEM)	N	%Stained	Persis. (d)	Mean Prewt. (mg/worker)
		1.00	8.00±9.27b	4	0	2-4	1.95±0.17b
		2.00	3.25±6.50b	4	0	2-4	1.85±0.16b

[1]Mean survival out of a total of 20 termites
[2]Direct Orange, ANOVA on Mean Survival, ns, (df=54, F=1.11, p<0.3292)
[3]Direct Orange, ANOVA on Mean Preweight, ns, (df=167, F=0.24, p<0.9618)

Table 3-6. The mean survival (± SEM), percentage stained at test termination, days of stain persistence, and the mean preweight (± SEM) of Reticulitermes spp. workers after staining with the anionic triphenylmethane stain: Fast Green FCF, at all concentrations tested

Stains	Other Names	Conc. (wt.vol)	Mean Survival[1] (± SEM)	N	%Stained	Persis. (d)	Mean Prewt. (mg/worker)
Fast Green FCF	Food Green	H20	18.46±1.66[2]	13	0	0	2.29±0.52[3]
C. I. 42053	3	0.05	18.75±1.48	12	44.3	7-15	2.20±0.45
		0.10	18.81±0.87	11	59.5	8-15	2.23±0.47
		0.25	19.16±1.33	12	62.5	14-15	2.22±0.46
		0.50	18.75±1.28	12	64.2	14-15	2.24±0.49
		1.00	18.16±1.32	6	57.8	14-15	2.05±0.34
		2.00	18.33±1.36	6	58.2	14-15	2.03±0.34

[1] [2]Mean survival out of a total of 20 termites
[3]Fast Green FCF, ANOVA on Mean Survival, ns, (df=71, F=0.54, p<0.7734)
[4]Fast Green FCF, ANOVA on Mean Prweight, ns, (df=221, F=1.06, p<0.3904)

Table 3-7. The mean survival (± SEM), percentage stained at test termination, days of stain persistence, and the mean preweight (± SEM) of Reticulitermes spp. workers after staining with the cationic azine stains: Janus Green B, Neutral Red, and Safranin O, at all concentrations tested

Stains	Other Names	Conc. (wt.vol)	Mean Survival[1] (±SEM)	N	%Stained	Persis. (d)	Mean Prewt. (mg/worker)
Janus Green B	Diazin	H2O	19.00±1.41a	4	0	0	2.47±0.63[2]
C. I. 11050	Green S	0.05	18.00±1.82a	4	20.7	7-15	2.20±0.47
		0.10	17.50±2.08a	4	35.8	7-15	2.22±0.46
		0.25	16.25±1.70ab	4	34.2	8-15	2.12±0.38
		0.50	11.00±5.29b	3	19.6	7-15	2.01±0.42
Neutral Red	—	H2O	19.00±1.41a	2	0	0	2.77±0.20ab
C. I. 50040		EtOH	19.50±0.70a	2	0	0	2.90±0.10a
		0.05	18.00±0.00a	2	83.3	15	2.71±0.09ab
		0.10	19.00±1.41a	2	100.0	15	2.64±0.09ab
		0.25	18.00±1.41a	2	100.0	15	2.60±0.16b
		0.50	19.00±0.00a	1	100.0	15	2.54±0.20b

Table 3-7. Cont.

Stains	Other Names	Conc. (wt.vol)	Mean Survival (±SEM)	N	%Stained	Persis. (d)	Mean Prewt. (mg/worker)
		1.00	0.00±0.00b	2	0	13	2.07±0.20c
		2.00	0.00±0.00b	2	0	5-13	2.08±0.08c
Safranin O	Basic Red 2	H20	15.00±7.92a	10	0	0	2.35±0.48a
C. I. 50240		EtOH	18.00±± 2.74a	10	0	0	2.34±0.47a
		0.05	18.40±1.71a	10	66.5	7-15	2.29±0.41a
		0.10	14.00±4.80a	10	98.4	15	2.23±0.37ab
		0.25	9.12±9.81ab	8	36.2	3-15	2.15±0.24ab
		0.50	8.62±7.85ab	8	41.1	5-15	2.10±0.26ab
		1.00	0.25±1.50b	4	25.0	4-15	2.12±0.31ab
		2.00	0.00±0.00b	4	0	3-4	1.88±0.13b

[1]Mean survival out of a total of 20 termites
[2]Janus Green B, ANOVA on Mean Preweight, ns, (df=59, F=1.45, p<0.2304)

Table 3-8. The mean survival (± SEM), percentage stained at test termination, days of stain persistence, and the mean preweight (± SEM) of Reticulitermes spp. workers after staining with the cationic thiazine stains: Methylene Blue, and Toluidine Blue, at all concentrations tested

Stains	Other Names	Conc. (wt.vol)	Mean Survival[1] (± SEM)	N	%Stained	Persis. (d)	Mean Prewt. (mg/worker)
Methylene	Basic Blue	H20	19.50±1.00a	4	0	0	2.65±0.19a
Blue	9	EtOH	18.75±2.50ab	4	0	0	2.65±0.11a
C. I. 52015		0.05	19.75±0.50a	4	0	0-9	2.58±0.19a
		0.10	18.25±0.50ab	4	2.8	6-15	2.26±0.19bc
		0.25	18.25±1.50ab	4	0	10-15	2.41±0.15ab
		0.50	18.00±1.41ab	4	0	13-14	2.21±0.20bc
		1.00	16.25±2.62ab	4	0	10-14	2.14±0.22c
		2.00	11.50±8.34b	4	7.8	10-15	1.87±0.28d
Toluidine	Basic Blue	H2O	18.00±1.41a	4	0	0	1.91±0.09a
Blue	17	EtOH	19.00±0.00a	4	0	0	1.78±0.05b
C. I. 52040		0.05	17.50±0.70ab	4	100.0	15	1.62±0.05c
		0.10	12.00±1.41b	4	100.0	15	1.46±0.03d
		0.25	3.00±2.82c	4	100.0	15	1.37±0.08de
		0.50	0.00±0.00c	4	0	8-9	1.29±0.06e

[1]Mean survival out of a total of 20 termites

Table 3-9. The mean survival (± SEM), percentage stained at test termination, days of stain persistence, and the mean preweight (± SEM) of Reticulitermes spp. workers after staining with the neutral diszao stain: Oil Red O, at all concentrations tested

Stains	Other Names	Conc. (wt.vol)	Mean Survival[1] (± SEM)	N	%Stained	Persis. (d)	Mean Prewt. (mg/worker)
Oil Red O	Solvent Red	H_2O	19.37±0.74a	8	0	0	2.23±0.50b
C. I. 26125	27	Acetone	18.62±0.91a	8	0	0	2.26±0.50b
		0.0125	19.00±1.41a	2	0	10	3.15±0.11a
		0.025	18.50±1.00a	4	0	8-13	2.41±0.65b
		0.05	18.14±1.95a	7	57.1	8-15	2.27±0.53b
		0.10	17.62±3.33a	8	50.0	12-15	2.26±0.47b
		0.25	12.37±7.80ab	8	62.5	13-15	2.18±0.46b
		0.50	8.00±7.84b	6	33.3	2-15	1.93±0.21b
		1.00	4.50±6.13b	4	50.0	3-15	1.89±0.10b

[1]Mean survival out of a total of 20 termites

Table 3-10. The mean survival (± SEM), percentage stained at test termination, days of stain persistence, and the mean preweight (± SEM) of Reticulitermes spp. workers after staining with Reactive Green, at all concentrations tested

Stains	Other Names	Conc. (wt.vol)	Mean Survival[1] (± SEM)[2]	N	%Stained	Persis. (d)	Mean Prewt. (mg/worker)
Reactive Green	---	H20	18.33±3.20	6	0	0	2.14±0.08a
C. I. ---		0.05	19.50±0.83	6	0	2-4	2.17±0.06a
		0.10	19.66±0.51	6	0	3-5	2.17±0.05a
		0.25	19.20±0.83	5	0	3-5	2.18±0.10a
		0.50	19.60±0.5	5	2.1	3-4	2.16±0.09a
		1.00	19.40±0.89	5	0	3-4	2.14±0.09a
		2.00	17.66±1.03	6	0	3-4	1.96±0.13b

[1]Mean survival out of a total of 20 termites
[2]Reactive Green Mean Survival ANOVA ns (df=38, F=1.57, p<0.1869)

Table 3-11. The mean survival (± SEM), percentage stained at test termination, days of stain persistence, and the mean preweight (± SEM) of Reticulitermes spp. workers after staining with azo stains: Sudan IV and Sudan Orange, at all concentrations tested

Stains	Other Names	Conc. (wt.vol)	Mean Survival[1] (± SEM)	N	%Stained	Persis. (d)	Mean Prewt. (mg/worker)
Sudan IV	Scarlet	H20	19.50±1.00a	4	0	0	2.42±0.64[2]
C. I. 26105	Red,	Acetone	18.75±0.95ab	4	0	0	2.44±0.67
(Disazo)	Solvent	0.05	19.25±0.95a	4	93.4	15	2.42±0.66
	Red 24	0.10	17.00±1.63ab	4	100.0	15	2.40±0.67
		0.25	16.75±1.70ab	4	100.0	15	2.38±0.57
		0.50	17.25±1.50ab	4	100.0	15	2.30±0.60
		1.00	13.50±5.32b	3	95.0	15	2.28±0.58
		2.00	15.66±1.52ab	4	100.0	15	2.45±0.68
Sudan Orange	Solvent	H20	20.00±0.00a	4	0	0	2.13±0.06a
C. I. 11920	Orange 1	Acetone	20.00±0.00a	4	0	0	2.13±0.09a
(Monoazo)		0.05	18.33±1.15a	3	0	2-3	1.99±0.05b
		0.10	18.33±2.08a	3	0	2-3	1.88±0.07c

Table 3-11. Cont.

Stains	Other Names	Conc. (wt.vol)	Mean Survival (± SEM)	N	%Stained	Persis. (d)	Mean Prewt. (mg/worker)
		0.25	7.50±5.80b	4	0	2-3	1.79±0.06c
		0.50	5.50±6.55b	4	0	2-3	1.67±0.05d
		1.00	2.25±2.63b	4	0	2-3	1.66±0.04d
		2.00	0.00±0.00b	4	0	2-3	1.64±0.10d

[1]Mean survival out of a total of 20 termites
[2]Sudan IV, ANOVA on Mean Preweight, ns, (df=95, F=0.11, p<0.9973)

Table 3-12. The mean survival (± SEM), percentage stained at test termination, days of stain persistence, and the mean preweight (± SEM) of Reticulitermes spp. workers after staining with the cationic diphenylnaphthylmethane stain: Victoria Blue, at all concentrations tested

Stains	Other Names	Conc. (wt.vol)	Mean Survival[1] (± SEM)	N	%Stained	Persis. (d)	Mean Prewt. (mg/worker)
Victoria Blue	Basic Blue	H2O	15.50±6.36[1]	2	0	0	1.83±0.05a
R	11	EtOH	15.50±6.36	2	0	0	1.83±0.07a
C. I. 44045		0.25	10.00±14.14	2	0	10	1.58±0.10 b

[1]Mean survival out of a total of 20 termites
[2]Victoria Blue, ANOVA on Mean Survival, ns, (df=5, F=0.22, p<0.8178)

CHAPTER 4
LABORATORY EVALUATION OF FOOD PLACEMENT AND FOOD TYPES ON THE FEEDING PREFERENCE OF R. VIRGINICUS

Introduction

Studying the nutritional ecology of termites can be directly tied to applications such as the current bait technology for subterranean termite control (Su & Scheffrahn 1993b). The presence of preferred alternate food sources in the field, made establishing underground monitoring stations with wooden bait blocks difficult (see Chapter 2). The paradigm of nutritional ecology (Slansky 1982, Slansky & Rodriguez 1987) can become a component in a model of patch choice for Reticulitermes spp. Testing hypotheses in nutritional ecology requires a laboratory bioassay which can measure feeding preferences without results being artifacts of the bioassay design. Subterranean termite feeding tests are conducted as no-choice or choice tests. In no-choice tests, materials are placed in separate containers, allowing termites to feed only on one material. This method is an efficient way to screen chemicals for wood preservative treatments (ASTM 1972, Grace et al. 1992, Grace & Yamamoto 1992, Grace et al. 1993a, Su 1993c).

However, no-choice tests do not adequately assess feeding preference. When necessary, termites can feed on less preferred food in order to sustain

themselves. Smythe and Carter (1970) tested the feeding response of R. virginicus to wood of 11 tree species. Their results demonstrated that the rank of preference for five of the species increased when termites were allowed to feed in a choice versus a no-choice situation. These results indicate that feeding in a no-choice situation does not necessarily reflect that which occurs in a choice test.

Choice tests allow termites to forage between the control and test substrate in the same container. Usually, materials are placed at opposite ends of the foraging arena to avoid the effects of possible leaching between food materials. If several materials are to be tested, substrates are arranged in a circular fashion, randomly and evenly spaced around the perimeter of the container, where relative consumption is a measure of preference. Choice tests are useful to measure feeding deterrence for bait toxicants (Su & Scheffrahn 1988c, 1989a, b, 1993b).

Consumption is affected by a complex of factors (Delaplane 1991), including the population density of the termites (Esenther 1980b, Lenz & Williams 1980, Lenz & Barrett 1984), termite vigor due to colony origin (Su & La Fage 1984a, Lenz 1985), feeding material (Smythe & Carter 1970, Behr et al. 1972, Howard & Haverty 1979, Su & Tamashiro 1983), previous damage by conspecifics (Delaplane & La Fage 1989b), temperature (Haverty & Nutting 1974), moisture (Delaplane & La Fage 1989a), and mortality over time (Su & La Fage 1984b). In this study, the effect of food placement on consumption was

examined to address the questions: Does food placement affect food choice and does the random search for food result in random feeding? The efficiency of two bioassay designs using two different food types in a laboratory choice test and the effect of time on consumption was examined within and between experimental units in order to determine the optimal time interval to run food preference tests under our conditions.

Materials and Methods

Termites. Two colonies of R. virginicus were field collected from the University of Florida Horticulture Unit and Animal Science Unit in Gainesville, Florida. Infested logs were cut into transportable sections, brought back to the laboratory, and held in containers (55 cm diam., 70 cm high). Several layers of moistened cardboard were wedged between two pieces of log. The container was then covered overnight to allow the termites to invade the cardboard. Cardboard sandwiched between pieces of log yielded significantly more termites than moistened cardboard simply laid across the infested wood (La Fage et al. 1983).

Thousands of termites have been retrieved with the method of La Fage et al. (1983), probably because log infestations were high. However, more than 50% of the time, only 50-100 termites were retrieved from our logs with La Fage's system, probably because log infestations were moderate. Termites were gently knocked from the cardboard pieces into a clean metal tray and isolated from debris by allowing them to walk on a wooden ramp (Tamashiro et

al. 1973) or by allowing them to cling sheets of moistened filter paper. Termites were then tapped off into a clean container.

Food. Approximately 2 grams of cardboard or filter paper (Whatman No. 2) were pulped in a blender for 20-30 s in approximately 250 ml of tap water. Water was evacuated under vacuum from the pulp using a Büchner funnel lined with filter paper (Whatman No. 2). The cardboard or filter paper patty produced was cut into 1.0-1.5 cm squares (2 to 3 mm thick), dried for a minimum of 24 h, at 60°C, and weighed (± 0.01 mg). Each food piece weighed between 50 and 80 (± 0.1 mg).

Assay arena. Approximately 10 cc of acetone-washed, oven-dried sand was placed in a glass Petri dish (100 mm diam., 15 mm high) as a foraging matrix. The sand was evenly moistened with 5 ml distilled water. Cardboard or filter paper squares moistened to saturation with distilled water were positioned in either the paired or the split design. The distance between food pieces in the split design was about 4 cm; food pieces in the paired design were adjacent to each other.

Food Placement. In order to test the effect of food placement on consumption, two pieces of the same food type (i. e., cardboard-only or filter paper-only) were placed into an assay arena in either the split or paired design. Each food piece was designated as "piece 1" or "piece 2."

One-hundred fifty worker termites were introduced into each arena and allowed to feed for intervals of 24, 48, 72, 96, or 120 h. Ten experimental units

(EUs) were assembled for each time interval (5), food type (2), placement combination (2), and colony (2), for a total of 400 EUs. After termite feeding, remnants of cardboard or filter paper pieces were rinsed clean, oven dried as previously described, and re-weighed. Consumption was determined by subtracting pre- and post-weights of the cardboard or filter paper.

Data analysis. consumption on one piece was hypothesized to be equal to consumption on the alternate piece (H_O: $\mu_1=\mu_2$) if averaged over all experimental units, because equivalent food types were used. Unordered data for cardboard-only and filter paper-only combinations were analyzed separately using a paired t-test (SAS Institute 1985). Feeding time intervals which achieved no significant difference in consumption between food pieces were determined to be the optimal feeding times to be used in preference tests.

Lack of sigificance for the paired t-test could be the result of the termites feeding equally on each piece, or the nullifying effect of heavy consumption on "piece 1", but not "piece 2," and vice versa, so that the sum of the difference value in the paired t-test was small. In order to further investigate the question of whether feeding was equal within an experimental unit over time, the more consumed piece was labeled "piece 1." The alternate piece was labeled "piece 2." Assuming random selection of food within the arena would result in random feeding, consumption on "piece 1" was hypothesized to be equal to consumption on "piece 2," within each placement pattern (paired or split) for each food type

(cardboard-only and filter paper-only). The ordered data were again analyzed with a paired t-test.

Lastly, consumption variances for each food type were hypothesized to be equal, regardless of placement (i. e., testing between treatment variability). Non-homogeneous variances would indicate that the termites were foraging and feeding differently, depending on whether food was placed in the paired or split design. Unordered data were analyzed using Hartley's F_{max} test for homogeneity of variances, where F_{max} was calculated as the larger variance of either food type over the smaller variance (Sokal & Rohlf 1981).

Food type assay. To compare the power of the bioassay designs, preference of cardboard or filter paper by Retciulitermes spp. was documented. Ten EU's were assembled for each combination of time (5), colony (2), and food placement (2), for a total of 200 EUs. Data were collected in the same manner as the food placement assay.

The null hypothesis was that within each placement design, there would be no difference in consumption between cardboard and filter paper (H_o: $\mu_{CB}=\mu_{FP}$). The minimum difference in consumption to be detected (described below), was calculated and 30 more EUs in the paired configuration at 72 h were assembled to verify the calculated sample size required to achieve significant differences in consumption. Data from the paired and split designs were analyzed separately using a paired t-test (SAS Institute 1985). The cardboard

only and filter paper only combinations of the food placement assay served as controls.

Efficiency of the bioassay. An efficient design was defined as one that would correctly detect significant consumption differences using the least number of EUs. Efficiency of the design was determined by calculating the sample size needed to detect the desired smallest true difference, while maintaining a power of 0.90 and 0.80. The power of the test (1-β) is the ability to reject the null hypothesis, H_o, when H_o is false. If we assume H_o: $\mu_{CB}=\mu_{FP}$, where μ_{CB} is the mean consumption on cardboard, and μ_{FP} is the mean consumption on filter paper, and H_1: $\mu_{CB}\neq\mu_{FP}$, where consumption means are not equal, then the smallest true difference we desire to detect would be, δ, where $\delta=\mu_{CB}-\mu_{FP}$. Sample sizes were calculated using a program code for SAS developed by G. Vining (University of Florida, Department of Statistics) based on the non-centrality parameter described in Montgomery (1984). An estimate of the ratio of δ to the variance, σ^2, was required to produce the sample size. The smallest true difference (δ) of food pieces in the paired design was compared with σ_{PR}^2 of the paired design, and σ_{SP}^2 of the split design, respectively.

Results

Food placement. Results of the paired t-test using unordered data indicated significant differences in consumption between cardboard pieces in the paired design at 24 and 48 h (Figure 4-1A), but there were no significant differences in the split design for all time intervals (Figure 4-1B). There were no significant

differences in consumption of the filter paper pieces in the paired design at any time intervals (Figure 4-2A). However, there were significant differences in consumption between filter paper pieces in the split design at 48 and 72 h (Figure 4-2B). Results of the paired t-test using ordered data indicated significant differences in consumption within EUs at every time interval, for both food types, in the paired and split designs (Figures 4-3 & 4-4).

Consumption variances for the unordered data of the split design were significantly greater than variances of the paired design for the cardboard-only combination at all time intervals (Table 4-1). Consumption variances of the split design became significantly greater at 96 and 120 h for the filter paper-only combination (Table 4-1). Calculated F_{max} values for time intervals 24, 48, and 72 h in the filter paper-only combination were close to the critical value (F_{max}>2.46) for significance.

Food Type. There was no significant difference at α=0.05, between consumption on cardboard or filter paper in the paired design choice test when N=20, at 48, 96, and 120 h, although cardboard was consistently preferred over filter paper (Figure 4-5A). In the case where the sample size approached the calculated sample size of n=55 (Table 4-2) in the paired design at 72 h, cardboard was significantly more consumed over filter paper (Figure 4-5A). In the split design, cardboard was significantly preferred over filter paper at each time interval (Figure 4-5B).

Efficiency of the bioassay. Since cardboard was consistently preferred, the smallest true difference selected for sample size calculations was $\delta_{PR}=\mu_{CB}-\mu_{FP}$, where δ_{PR} was the difference in consumption between cardboard and filter paper in the paired design. Although a stronger difference in consumption between cardboard and filter paper existed in the split design, the sample size required to detect δ_{PR} became large due to the large variance in feeding in the split design. In general, the sample size required to achieve significance increased as time increased for both the paired and the split designs (Table 4-2).

Discussion

At most time intervals in the food placement assay, consumption of equivalent food types was equal over the average of experimental units when data were not ordered. However, there appears to be a propensity of termites to feed at a single site, so that significant differences for unordered data in consumption between filter paper pieces at 48 and 72 h is probably the result of insufficient sample size. The propensity of termites to feed at one site is emphasized when data were ordered. Results of the ordered data strongly suggests that termites may search for food randomly, but feeding was not random under these conditions, perhaps as a result of recruitment to an acceptable food source. The tendency for one piece to be more heavily consumed over another in assays such as the split design supports the results of Delaplane & La Fage (1987). They found that C. formosanus did not feed

equally on wood blocks of equivalent type when blocks were placed in foraging chambers equidistant (9.8 cm) from a central chamber where the termites were released.

A feeding assay which indicates significant differences in consumption when food materials are equivalent, may also bias feeding preferences when food of different types are present. If searching for food is assumed to be random, but feeding is biased toward one food source, then food placement becomes an important factor affecting consumption in choice tests. The paired design may overcome the propensity of termites to feed most heavily at one food source after 72 h, because there were no significant differences in consumption between food pieces in either the cardboard (Figure 4-1A) or the filter paper (Figure 4-2B) controls for food quantities of 50 to 80 mg, when 150 termites were used in each EU.

Time in relation to the quantity of food available was also an important factor in constructing preference tests. If preference is defined as increased consumption due to a process which includes orientation to a particular material (Delaplane & La Fage 1989c, Grace 1991b), then termites must be allowed sufficient time to forage through a matrix; otherwise, the increased consumption of one substrate over another may not reflect preference, but simply a lack time allowed for the termites to choose between substrates. If termites are allowed to feed for a time interval past the depletion of the preferred food source so that termites are forced to feed on the less preferred source or else starve, again

preferences can be misinterpreted. In a short-term feeding test, the paired design may decrease the variability in termite feeding due to orientation effects because termites need only orient toward food types placed in one location, then choose between the two types, as opposed to choosing between two orientations (split design), and then choosing between two food types.

Termites were also shown to prefer cardboard over filter paper for all time intervals. Although a stronger difference in consumption between the cardboard and filter paper existed in the split design (unordered data), significant differences in the filter paper control at 48 and 72 h (Figure 4-2B) make the split design a poor choice for a preference bioassay. The difference in consumption between cardboard and filter paper at 72 h was 7.6 mg in the paired design. The difference in consumption between the filter paper and cardboard controls were 0.9 and 3.2 mg, respectively. The smaller difference in consumption in the controls allowed for more sensitive detection of differences in treatment units. To obtain the same level of sensitivity in consumption differences in the split design, a sample size of 67 or 89 would be required to maintain a power of 0.8 or 0.9, because of the large variance involved.

If termites are allowed to choose a food type placed in the split design, recruitment may be a consideration. Termites may search for food randomly, but once food is found, termites may be recruited back to a food source by cues not investigated in this study. Recruitment to a food source should not be equated with food quality in this study.

In the split design, termites in the food type study located both cardboard and filter paper pieces, but congregated and fed most heavily under the cardboard piece, perhaps indicating recruitment to cardboard. In the paired design, the preference of the cardboard as a food source may have been diminished when termites, which are generalist cellulose feeders, were presented with another acceptable food source, filter paper. Thus consumption differences were not as extreme.

Understandably, there are times when the paired design may not be feasible because the materials to be tested leach, causing contamination of substrates. In these cases, the wide variance produced by the split design may result in feeding preferences which are simply an artifact of too few replications. One remedy to the wide variance produced in the split design would be to allow foraging times which would produce no significant differences in controls and to use a sufficiently large number of replicates.

Table 4-1. F_{max} values for variances from consumption by R. virginicus in the paired or split design

Time	Food	Design	Variance	F_{max}
24	CB/CB	PR	7.419	3.529**[1]
		SP	26.183	
48	CB/CB	PR	24.236	4.605**
		SP	111.610	
72	CB/CB	PR	35.499	7.915**
		SP	280.965	
96	CB/CB	PR	58.562	3.900**
		SP	228.384	
120	CB/CB	PR	70.064	6.701**
		SP	469.485	
24	FP/FP	PR	10.695	2.402
		SP	25.691	
48	FP/FP	PR	48.522	2.041
		SP	99.061	
72	FP/FP	PR	107.922	2.391
		SP	258.036	
96	FP/FP	PR	113.875	4.387**
		SP	499.533	
120	FP/FP	PR	213.062	2.862*[2]
		SP	609.526	

[1]Significant at α=0.01, if F_{max}, F>3.32.
[2]Significant at α=0.05, if F_{max}, F>2.46.

Table 4-2. Sample sizes required to achieve significant differences in consumption by R. virginicus at α=0.05 and maintain powers of 0.8 or 0.9 for $\delta_{PR} = \mu_{CB} - \mu_{FP}$

	Power 0.8		Power 0.9	
Time	N'[1] Paired	N' Split	N' Paired	N' Split
24	19	14	25	18
48	63	56	84	75
72	55	67	73	89
96	71	158	95	211
120	81	223	107	298

[1]N' is the calculated sample size.

Figure 4-1. Consumption differences for R. virginicus, p-values, and t-values of cardboard pieces in the food placement assay using unordered data. (A) Paired. (B) Split.

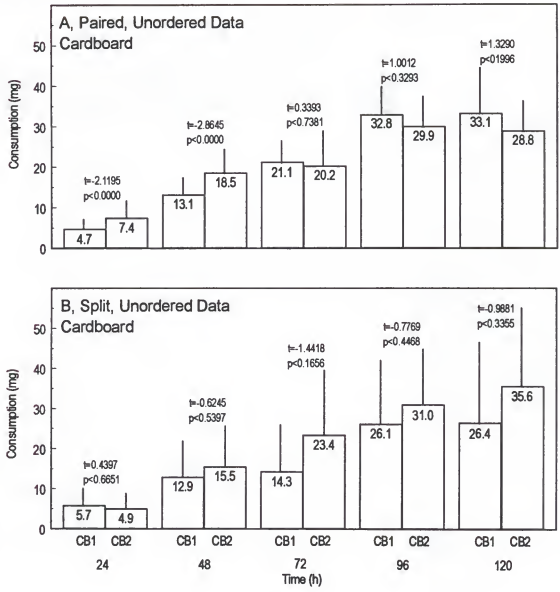

A, Paired, Unordered Data
Cardboard
Consumption (mg)
t=-2.1195
p<0.0000
4.7
7.4
t=-2.8645
p<0.0000
13.1
18.5
t=0.3393
p<0.7381
21.1
20.2
t=1.0012
p<0.3293
32.8
29.9
t=1.3290
p<01996
33.1
28.8
B, Split, Unordered Data
Cardboard
Consumption (mg)
t=0.4397
p<0.6651
5.7
4.9
t=-0.6245
p<0.5397
12.9
15.5
t=-1.4418
p<0.1656
14.3
23.4
t=-0.7769
p<0.4468
26.1
31.0
t=-0.9881
p<0.3355
26.4
35.6
CB1 CB2
24
48
72
96
120
Time (h)

Figure 4-2. Consumption differences for R. virginicus, p-values, and t-values of filter paper pieces in the food placement assay using unordered data. (A) Paired. (B) Split.

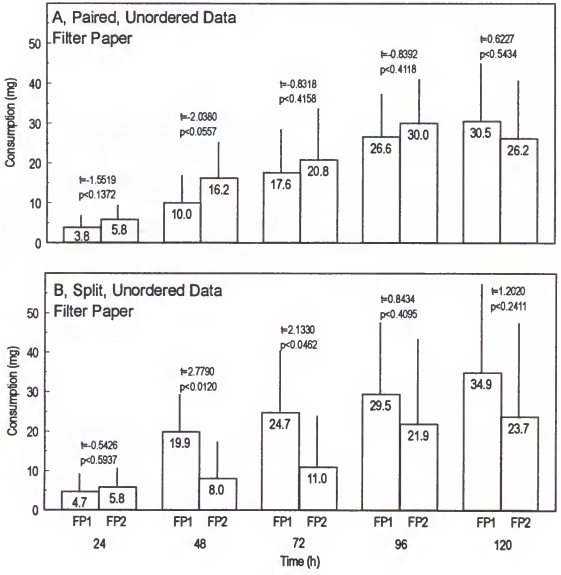

A, Paired, Unordered Data
Filter Paper
Consumption (mg)
t=-1.5519
p<0.1372
3.8
5.8
t=-2.0380
p<0.0557
10.0
16.2
t=-0.8318
p<0.4158
17.6
20.8
t=-0.8392
p<0.4118
26.6
30.0
t=0.6227
p<0.5434
30.5
26.2
B, Split, Unordered Data
Filter Paper
Consumption (mg)
t=-0.5426
p<0.5937
4.7
5.8
t=2.7790
p<0.0120
19.9
8.0
t=2.1330
p<0.0462
24.7
11.0
t=0.8434
p<0.4095
29.5
21.9
t=1.2020
p<0.2411
34.9
23.7
FP1 FP2
24
FP1 FP2
48
FP1 FP2
72
FP1 FP2
96
FP1 FP2
120
Time (h)

Figure 4-3. Consumption differences for R. virginicus, p-values, and t-values of cardboard pieces in the food placement assay using ordered data. (A) Paired. (B) Split.

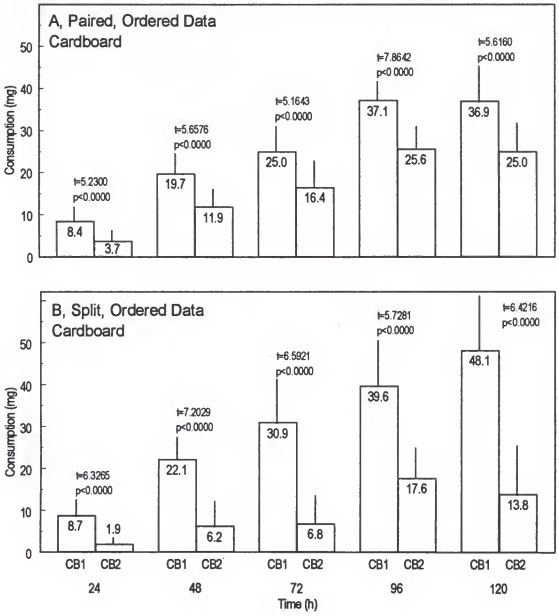

A, Paired, Ordered Data
Cardboard
Consumption (mg)
t=5.2300
p<0.0000
8.4
3.7
t=5.6576
p<0.0000
19.7
11.9
t=5.1643
p<0.0000
25.0
16.4
t=7.8642
p<0.0000
37.1
25.6
t=5.6160
p<0.0000
36.9
25.0
B, Split, Ordered Data
Cardboard
Consumption (mg)
t=6.3265
p<0.0000
8.7
1.9
t=7.2029
p<0.0000
22.1
6.2
t=6.5921
p<0.0000
30.9
6.8
t=5.7281
p<0.0000
39.6
17.6
t=6.4216
p<0.0000
48.1
13.8
CB1 CB2
24
48
72
96
120
Time (h)

Figure 4-4. Consumption differences for R. virginicus, p-values, and t-values of filter paper pieces in the food placement assay using ordered data. (A) Paired. (B) Split.

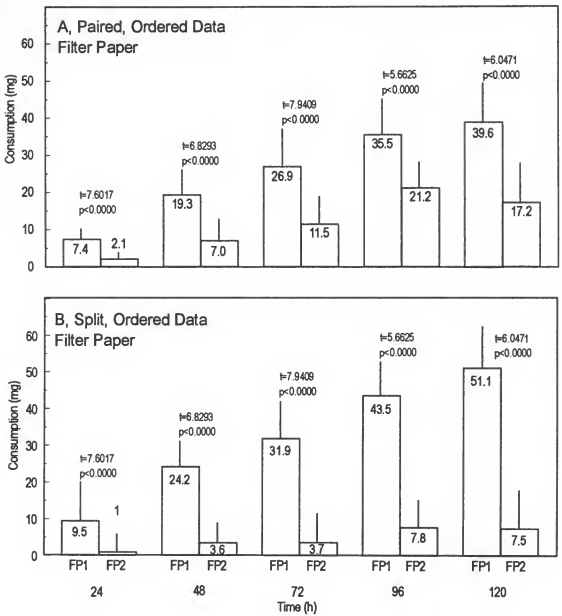
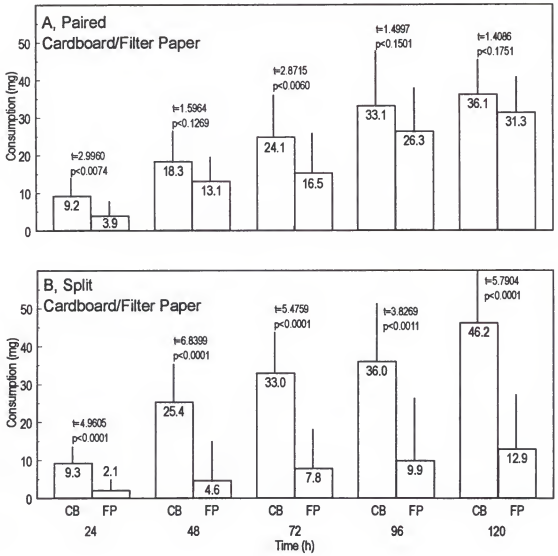

A, Paired, Ordered Data
Filter Paper
Consumption (mg)
60
50
40
30
20
10
0
t=7.6017
p<0.0000
7.4
2.1
t=6.8293
p<0.0000
19.3
7.0
t=7.9409
p<0.0000
26.9
11.5
t=5.6625
p<0.0000
35.5
21.2
t=6.0471
p<0.0000
39.6
17.2
B, Split, Ordered Data
Filter Paper
Consumption (mg)
60
50
40
30
20
10
0
t=7.6017
p<0.0000
9.5
1
t=6.8293
p<0.0000
24.2
3.6
t=7.9409
p<0.0000
31.9
3.7
t=5.6625
p<0.0000
43.5
7.8
t=6.0471
p<0.0000
51.1
7.5
FP1 FP2 FP1 FP2 FP1 FP2 FP1 FP2 FP1 FP2
24 48 72 96 120
Time (h)

Figure 4-5. Consumption for R. virginicus of cardboard and filter paper in choice tests. (A) Paired design. (B) Split design.


A, Paired
Cardboard/Filter Paper
Consumption (mg)
0
10
20
30
40
50
t=2.9960
p<0.0074
9.2
3.9
t=1.5964
p<0.1269
18.3
13.1
t=2.8715
p<0.0060
24.1
16.5
t=1.4997
p<0.1501
33.1
26.3
t=1.4086
p<0.1751
36.1
31.3
B, Split
Cardboard/Filter Paper
Consumption (mg)
t=4.9605
p<0.0001
9.3
2.1
t=6.8399
p<0.0001
25.4
4.6
t=5.4759
p<0.0001
33.0
7.8
t=3.8269
p<0.0011
36.0
9.9
t=5.7904
p<0.0001
46.2
12.9
CB FP CB FP CB FP CB FP CB FP
24 48 72 96 120
Time (h)

CHAPTER 5
THE EFFECT OF SOLDIER PROPORTION ON WOOD CONSUMPTION AND WORKER SURVIVAL FOR R. VIRGINICUS

Introduction

There are two classes of insect growth regulators (IGR's) being investigated for use in baits for termite control, in addition to other active ingredients. They are chitin synthesis inhibitors (CSI) and juvenile hormone analogs (JHA) and mimics (JHM) (Su & Scheffrahn 1990c). The most prominent example of a chitin synthesis inhibitor bait is hexaflumuron which acts by preventing workers from properly molting (Su & Scheffrahn 1993b). Juvenile Hormone Analogs and JHMs cause an increase in soldier and presoldier formation with a subsequent decrease in workers, eventually leading to colony demise by disrupting the balance between castes (Hrdý & Krecek 1972, Haverty 1977, Haverty & Howard 1980, Haverty et al. 1989, Su & Scheffrahn 1989b, 1990c).

One reason increasing soldier proportions can cause colony demise is because soldier mandibles are primarily designed for defense not for chewing on wood. Workers must feed the soldiers, thus making soldiers expensive to maintain in a colony. If there are more soldiers in a colony than workers can support, the colony will die. The average soldier proportion for R. virginicus in

the field is only 1.75% (Haverty & Howard 1981) which is probably the optimal soldier proportion. Termite soldier proportions are known to increase above optimal levels before swarming, ostensibly to protect the alate exit holes against predators (Wilson 1971). Since greater than optimal numbers of soldiers can occur naturally and workers can maintain them during the swarming period, the first objective of this study was to define the maximum supportable soldier proportion before colony demise. The second objective was to determine the length of time colonies can be supported at the maximum soldier proportion for Reticulitermes spp.

Lastly, just as the optimal soldier proportion is probably not the maximum supportable soldier proportion, the optimal rate of consumption by workers is not necessarily the maximum rate of consumption. Consumption by workers at the maximum soldier proportions which can be supported before colony demise probably represents the maximum consumption rate of workers. Thus, the third objective of this study was to determine the relationship between increasing soldier proportion and wood consumption. This study provided background information for pyriproxyfen laboratory (see Chapter 6) and field (see Chapter 7) tests on controlling R. virginicus.

Materials and Methods

Methods were modified from Su & La Fage (1987). Termites were collected from two field sites and stored in containers (55 cm diam., 70 cm high) for no more than one year. Mean worker weight (± 0.01 mg) was determined for

each colony by weighing five groups of 10 individuals. An experimental unit (EU) consisted of a glass screw-top jar (53 mm diam.), containing 30 cc sand moistened with distilled water. Sand was acetone washed and oven dried. One slash pine (Pinus elliottii Engelm var ellioti) wood block (2 X 2 X 2 cm) was nested in the sand. Pine blocks were oven dried for at least 48 h at 80°C and weighed (± 0.01 mg). Each EU contained 100 termites in the following soldier:worker proportion: 0:100, 1:99, 3:97, 5:95, 10:90, 15:85, & 20:80. These proportions corresponded to percent soldiers in a cohort as follows: 0, 1, 3, 5, 10, 15, & 20%. Experimental units were prepared in a randomized complete block design for each of the four blocks (7 proportions X 8 weeks X 3 replicates) for a total of 168 EUs. One colony was used three times because sufficient quantities of termites to complete one block could not be found from other sources during the time scheduled to conduct the experiment. Units were held in Florida Reach-In (Walker et al. 1993) environmental chambers at 28±1°C.

Three EUs from each proportion group were disassembled weekly for up to eight weeks. Surviving workers, soldiers, and presoldiers were counted and mean weights (± 0.01 mg) determined. Wood blocks were washed, oven dried as before, and reweighed (± 0.01 mg) as a measure of relative consumption. Consumption was calculated as mg wood/ g worker/ day (Su & La Fage 1984b).

Data analysis. Simple linear regression was done for each soldier proportion. Survival of workers and soldiers, were totaled, as the dependent variable; time was the independent variable. Variances were not homogeneous

and increased with time. Thus, survival was weighted by 1/variance of the total survival to the give the best linear unbiased estimator (Neter et al. 1990). Once regressions were determined to be significant, the hypothesis that soldier proportions would affect survival was tested by comparing the regression slope for soldier proportion 1, b_1, with slopes for all other soldier proportions (Montgomery 1984). Soldier proportion 1 was selected because this proportion most closely approximated the 1.75% soldier proportion found in the field (Howard & Haverty 1981).

The effect of soldier proportion on worker consumption was tested with analysis of variance, on unweighted and weighted data. Consumption was weighted as described above. If ANOVA on weighted data was significant, means were separated with Bonferroni's t-test to adjust for Type I error and unequal sample size (SAS Institute 1985).

Results and Discussion

Soldier proportion on survival. Regressions of survival on time for all soldier proportions were significant (Table 5-1) indicating a significant decrease in survival over time. Regressions for unweighted data were also significant; therefore, these data were interpreted with confidence. Comparisons between slopes for soldier proportion 1 and 10 (Figure 5-1B), and 1 and 20 (Figure 5-1D) were significantly different (Table 5-2). Unexpectedly, termite cohorts with 10% soldiers had a higher survival rate than termite cohorts with 1% soldiers. This result may be due to a lack of replication or the effects of Entomophthora

coronata (Costantin) Kevorkian infection in termite cohorts of 1% soldiers as discussed below. Termite cohorts with 20% soldiers survived at a rate significantly less than termite cohorts with 1% soldiers. There were no significant differences for all other comparisons (Table 5-2, Figure 5-1A, 5-1C).

Haverty and Howard (1981) determined that soldier proportions can vary between 1 and 3% (Haverty & Howard 1981, Howard & Haverty 1981, see Appendix B). They were unable to cause colony demise by causing soldier proportions to rise at high as 8.8%, which was 5 times the normal soldier proportion. Data from this study indicate that colony demise can occur at soldier proportions ≥20, which is 16.6 times the normal average soldier proportion of 1.75%.

Although a linear model can fit data for both soldier proportions 1 and 20, the data for soldier proportion 20 suggest that an almost sigmoid relationship exists for termite survival, where termite cohorts are able to sustain high numbers of soldiers for about 4 to 5 weeks before falling off dramatically in survival (Figure 5-1D). Unlike Su & La Fage (1987), who found that soldiers were cannibalized by workers with soldier proportions ≥50% in C. formosanus, there was no evidence of soldier cannibalization in an effort to restore normal soldier proportions in this study. Instead, R. virginicus with soldier proportions ≥ 20% repeatedly succumbed to fungal infection by E. coronata (determined by Drion Boucias, UF Entomology and Nematology Department, Insect Pathologist).

Entomophthora coronata is apparently harbored in the termites naturally and only converts to the virulent form under stress. In this study, stress can include being taken out of colony and placed in EUs or confining termites with less than optimal soldier proportions. This fungus also exists with dead wood and leaves (Prasertphon 1963a), where it utilizes polysaccarides for nutrition (Prasertphon 1963b). Termites either acquire the initial infection through ingestion or through cuticular penetration (Yendol & Paschke 1965), but E. coronata toxins are probably responsible for termite death during the early stages of mycosis (Yendol et al. 1968). This pathogen has almost identical relative humidity and temperature requirements as Reticulitermes spp. (Yendol 1968) and has caused mortality in other EUs which were excluded from data analysis in this study.

Soldier proportion on consumption. Analysis of variance on unweighted consumption data resulted in no significant effects for proportion by week interaction ($p<0.6163$), week ($p<0.0852$), or proportion ($p<0.9035$). However, ANOVA on weighted consumption data resulted in significance effects for proportion by week interaction ($p<0.0001$), so ANOVA was done for each week, testing the effect of soldier proportion on consumption. These results were interpreted with caution.

In general, consumption tended to increase with increasing soldier proportion (Figures 5-2C, 5-2D, 5-3A, 5-3B) after the first 2 wk (Figures 5-2A & 5-2B), until weeks 7 and 8 (Figures 5-3C & 5-3D), where high mortality began to

appear. More replication with different colonies of R. virginicus should be tested before attempting further correlation of soldier proportion with consumption. However, these data tend to agree with Su & La Fage (1987). They demonstrated that consumption by workers increased as soldier proportion increased to 30 to 50% of the total population in C. formosanus, but declined when soldier proportions exceeded 50%.

The average weighted consumption values for all time intervals indicated that workers of soldier proportion 15 from blocks 1 and 3 consumed more wood than workers of soldier proportion 1, but consumption decreased when soldier proportion increased to 20% (Figure 5-4). The decrease in consumption at soldier proportion 20 may indicate that the termite cohort exceeded its maximum supportable soldier proportion. Workers from cohorts of blocks 2 and 4 consistently consumed more wood at soldier proportion 20 than workers of soldier proportion 1 (Figure 5-4). Termites from cohorts of blocks 2 and 4 did not experience the increase and subsequent decrease in feeding than termites in blocks 1 and 3 and probably did not reach the maximum supportable soldier proportion, even at 20%.

Using pyriproxyfen impregnated wood blocks (150 ppm) in a choice bioassay to induce presoldier formation, the percentage of soldiers and presoldiers can be increased to 39.2% in as little as 4 wk (Su & Scheffrahn 1989b). Results from this study indicate that colony demise can be expected with the use of a pyriproxyfen if at least 20% of the colony population can be

transformed into soldiers or presoldiers. The feasibility of pyriproxyfen in baits for subterranean termite control may be limited by the number of foragers that consume sufficient quantities of active ingredient to cause presoldier formation and food preference, given that these termites can be generalist cellulose feeders. These questions are further investigated in Chapters 6 and 7. Although details have not been clarified on the amount of pyriproxyfen subterranean termites must ingest or the whether continuous feeding necessary to produce an excess of soldiers (see Chapter 6), Jones (1984) has found that colonies can be suppressed with fenoxycarb, a JHM, in the field. Su (1994) has also successfully suppressed or eliminated colonies with the CSI, hexaflumuron, in the field. These results indicate that sufficient numbers of forager can attack a bait station to negatively impact the colony.

Table 5-1. Regression slopes, R-squared, and level of significance for the effect of soldier proportion on total R. virginicus cohort survival

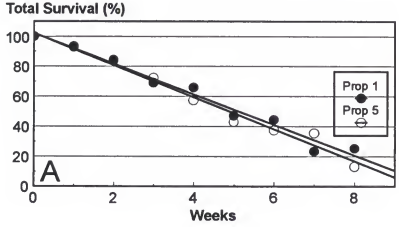
Proportion	R-squared	Slope±SE	Prob > |t|
0	0.9123	-11.3987±.0.7638	0.0001
1	0.8946	-12.0162±0.7807	0.0001
3	0.9655	-13.0716±0.4851	0.0001
5	0.9612	-11.8680±0.5892	0.0001
10	0.5253	-7.9473±1.4506	0.0001
15	0.9342	-11.9984±0.6855	0.0001
20	0.9801	-16.4963±0.9726	0.0001

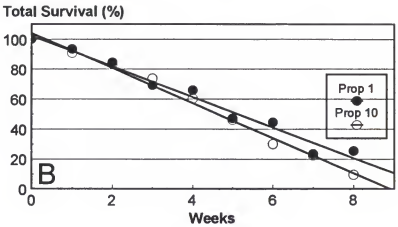
Table 5-2. Null hypotheses tested for each pair of regression slopes for R. virginicus cohort survival, where b_1 is the slope for soldier proportion 1, etc.

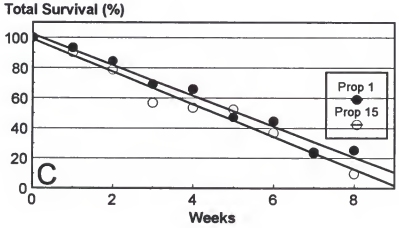
H_o	t-value
$b_1=b_0$	-0.7910
$b_1=b_3$	1.3519
$b_1=b_5$	-0.1898
$b_1=b_{10}$	-5.2119**[1]
$b_1=b_{15}$	0.0228
$b_1=b_{20}$	5.7386**[1]

[1]Significant at $p<0.05$

Figure 5-1. Simple linear regressions comparing survival for R. virginicus cohorts with the following soldier proportions: 1 & 5 (A), 1 & 10 (B) which are significantly different, 1 & 15 (C), 1 & 20 (D) which are significantly different.


Total Survival (%)
100
80
60
40
20
0
0
2
4
6
8
Weeks
Prop 1
Prop 5
A


Total Survival (%)
100
80
60
40
20
0
0
2
4
6
8
Weeks
Prop 1
Prop 10
B


Total Survival (%)
100
80
60
40
20
0
0
2
4
6
8
Weeks
Prop 1
Prop 15
C

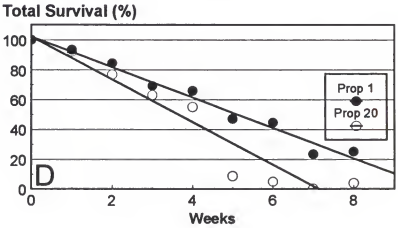
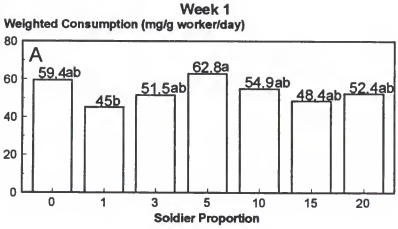
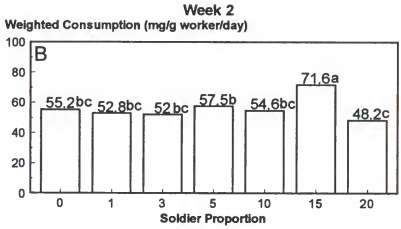
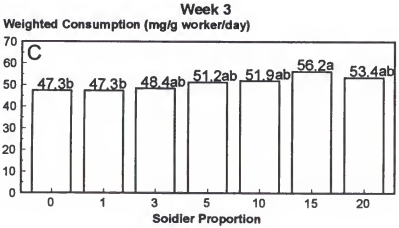

Total Survival (%)
100
80
60
40
20
0
0
2
4
6
8
Weeks
Prop 1
Prop 20
D

Figure 5-2 The weighted consumption of wood by R. virginicus workers for each soldier proportion at week 1, $p<0.0095$ (A); week 2, $p<0.0011$ (B); week 3, $p<0.0006$ (C); and week 4, $p<0.0001$ (D). Means separated by different letters indicate signficant differences using Bonferroni's t-test.


Week 1
Weighted Consumption (mg/g worker/day)
A
59.4ab
45b
51.5ab
62.8a
54.9ab
48.4ab
52.4ab
0
1
3
5
10
15
20
Soldier Proportion


Week 2
Weighted Consumption (mg/g worker/day)
B
55.2bc
52.8bc
52bc
57.5b
54.6bc
71.6a
48.2c
0
1
3
5
10
15
20
Soldier Proportion


Week 3
Weighted Consumption (mg/g worker/day)
C
47.3b
47.3b
48.4ab
51.2ab
51.9ab
56.2a
53.4ab
0
1
3
5
10
15
20
Soldier Proportion

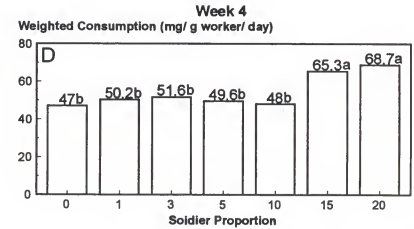
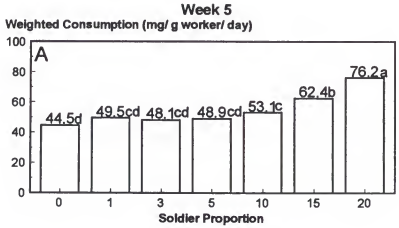
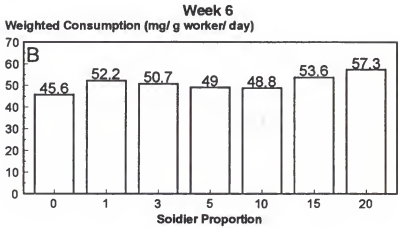
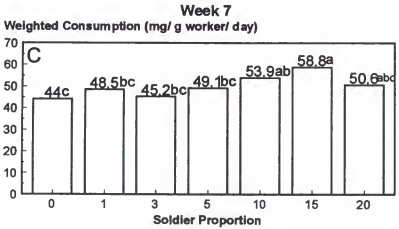

Week 4
Weighted Consumption (mg/ g worker/ day)
D
47b
50.2b
51.6b
49.6b
48b
65.3a
68.7a
0
1
3
5
10
15
20
Soldier Proportion

Figure 5-3. The weighted consumption of wood by R. virginicus workers for each soldier proportion at week 5, $p<0.0029$ (A); week 6, $p<0.5464$ (B); week 4, $p<0.0022$ (C); and week 8, $p<0.0001$ (D). Means separated by different letters indicate signficant differences using Bonferroni's t-test.


Week 5
Weighted Consumption (mg/ g worker/ day)
A
44.5d
49.5cd
48.1cd
48.9cd
53.1c
62.4b
76.2a
0
1
3
5
10
15
20
Soldier Proportion


Week 6
Weighted Consumption (mg/ g worker/ day)
B
45.6
52.2
50.7
49
48.8
53.6
57.3
0
1
3
5
10
15
20
Soldier Proportion


Week 7
Weighted Consumption (mg/ g worker/ day)
C
44c
48.5bc
45.2bc
49.1bc
53.9ab
58.8a
50.6abc
0
1
3
5
10
15
20
Soldier Proportion

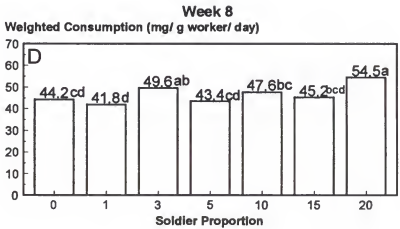
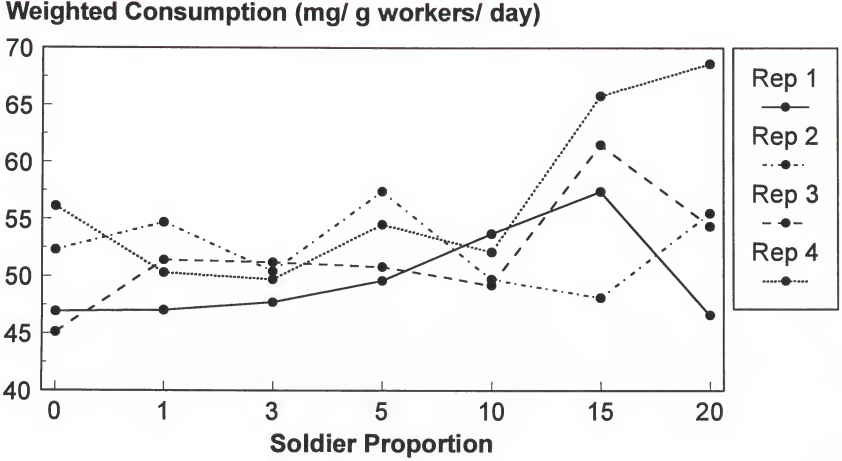

Week 8
Weighted Consumption (mg/ g worker/ day)
D
44.2cd
41.8d
49.6ab
43.4cd
47.6bc
45.2bcd
54.5a
0
1
3
5
10
15
20
Soldier Proportion

Figure 5-4. The weighted consumption of wood by R. virginicus workers for each soldier proportion group, averaged over eight weeks.


Weighted Consumption (mg/ g workers/ day)
70
65
60
55
50
45
40
0
1
3
5
10
15
20
Soldier Proportion
Rep 1
Rep 2
Rep 3
Rep 4

CHAPTER 6
LABORATORY EVALUATION OF CONTINUOUS AND INTERMITTENT FEEDING OF R. VIRGINICUS ON PYRIPROXYFEN

Introduction

The use of insect growth regulators (IGRs) for pest control has broad appeal because of the specificity to the target insect and minimum mammalian toxicity. For this reason, IGRs, either juvenile hormone analogs or mimics (JHAs/JHMs) and chitin synthesis inhibitors (CSIs), are being widely investigated for use in structural pest control on ants (Glancy et al. 1990, Banks & Lofgren 1991, Reimer et al. 1991, Williams & Vail 1993, Williams & Vail 1994), fleas (Hinkle et al. 1994), cockroaches (Brenner et al. 1988, Koehler & Patterson 1989, 1990; Kramer et al. 1989, Kramer et al. 1990), and termites (Howard & Haverty 1978, Haverty & Howard 1979, Howard 1980, Jones 1984, 1987, 1989, Haverty et al. 1989, Su & Scheffrahn 1989b, Su 1991, 1994; Su et al. 1991b).

The JHM, pyriproxyfen (Nylar, S31183, Sumitomo Chemical Company, Osaka, Japan), has been evaluated in the laboratory for control of the eastern (Su & Scheffrahn 1989b) and Formosan subterranean termites (Haverty et al. 1989, Su & Scheffrahn 1989b). An active ingredient which would require intermittent rather than continuous feeding may be more advantageous for field applications in termite control because issues of food quality and patch

choice as related to bait efficacy are unresolved. The purpose of this study was to evaluate the effect of continuos feeding for different lengths of time and intermittent feeding on pyriproxyfen, on the production of soldier intercastes of R. virginicus.

Material and Methods

Termites. Four colonies of R. virginicus were field collected in pine logs from the Horticulture Unit, the Bee Laboratory, and two areas across the Animal Science Unit, all of the University of Florida, Gainesville. Infested logs were cut into transportable sections, brought back to the laboratory and held in containers (55 cm diam., 70 cm high) for less than one month before use. Logs were broken into pieces and termites were knocked into plastic containers (16 X 20 cm, 8 cm high) which were sealed and held for 24 h to help ensure that injured termites were not used in the assay. After 24 h, moistened pieces of filter paper were placed in the plastic containers and termites were allowed to cling onto the filter paper, then gently knocked free onto a clean metal tray for counting.

Treatments. Corrugated cardboard pieces (5 cm wide, 30 cm long) were rolled, secured with a paper clip, and oven-dried for at least 24 h at 80°C. Dried cardboard rolls were weighed (± 0.1 mg) and treated with pyriproxyfen to yield final concentrations of 0 (acetone only), 50, 100, 150 ppm. The impregnation procedure was after Su et al. (1991b), except that cardboard rolls were soaked in various acetone-pyriproxyfen solutions for at least 5 s instead of being placed

under vacuum. Rolls were allowed to dry under a hood for a minimum of 24 h before use.

Treated cardboard rolls were placed in a glass screw-top jar (Qorpak, 53 cm diam). Approximately 70 cc of acetone washed-oven dried sand was poured around the cardboard roll as a foraging matrix. The sand and cardboard roll were moistened with approximately 13 cc of distilled water. One soldier and 99 workers were added to the jar. Termites were allowed to feed in two different phases for three different time intervals. In phase one, termites were allowed to feed continuously for 2 or 4 wk on treated cardboard, or intermittently, for 1 d on treated cardboard, then for 6 d on untreated cardboard. In phase two, termites were allowed to feed on untreated cardboard for another 4 wk.

Numbers of workers, presoldiers, and soldiers were recorded after continuous feeding on treated cardboard for 2 or 4 wk (phase one), and again after continuous feeding on untreated cardboard for 4 wk (phase two). Numbers of workers, presoldiers, and soldiers were recorded only after feeding on untreated cardboard for 4 wk (phase two) for the intermittent portion, because excessive handling would have caused high mortality. At the termination of feeding on untreated cardboard for 4 wk (phase two), the weights of termites in each caste were also recorded for continuous and intermittent feeding experiments.

Consumption was measured as cardboard weight loss ($\pm$ 0.1 mg) which was defined as the difference in the weight of cardboard before and after termite

feeding. Cardboard rolls were thoroughly dried and brushed clean. Cleaned cardboard rolls were oven dried as described above and re-weighed (± 0.1 mg).

Experimental design and data analysis. Each jar was considered one experimental unit (EU) in a randomized complete block design. Five EUs were prepared for each combination of concentration (4), time interval (3), and colony (4), including one set of controls without termites for a total of 300 EUs. Controls without termites were used to adjust consumption values on cardboard.

After feeding on treated cardboard (phase one), the sum of presoldiers and soldiers for each continuous interval was subject to analysis of variance. If ANOVA was significant, Bonferroni's t-test was done to check for differences in soldier/presoldier formation with increasing concentrations of pyriproxyfen. Bonferroni's t-test allows means separations to be done for cells with unequal sample size while adjusting for Type I error. The analysis was repeated for the sum of presoldier and soldiers after termites were allowed to feed on untreated cardboard for 4 wk (phase two). A paired t-test was also used to test for significant differences in the dependent caste numbers at the end of phases one and two. The sum of presoldiers and soldiers was transformed by log(x)+0.5 to linearize the data and create homogeneous variances. Data were then back-transformed for reporting purposes. The effect of presoldier formation on total survival was tested by ANOVA, followed by Bonferroni's t-test, if ANOVA was significant. Cardboard weight loss was analyzed with ANOVA, followed by Bonferroni's t-test, if necessary, for each time interval and concentration.

Results and Discussion

Analysis of variance was not significant for dependent caste formation after termites were allowed to feed on cardboard treated with the various concentrations of pyriproxyfen for 2 wk (Figure 6-1A). After phase two, ANOVA was significant ($p<0.0152$). Bonferroni's t-test indicated that termites which previously fed on cardboard rolls with 150 ppm had significantly higher numbers of presoldiers and soldiers in phase two than did termites which fed on cardboard treated with 0 or 50 ppm (Figure 6-1B). This result was mainly due to one colony which accounted for high numbers of presoldiers during phase two.

A paired t-test indicated that there was no significant difference in dependent caste formation between phases one and two (Table 6-1, Figure 6-1A, 6-1B) for termites which were allowed to feed continuously for 2 wk on pyriproxyfen, except at 50 ppm. At 50 ppm, presoldier and soldier numbers were significantly decreased (Figure 6-1A, 6-1B). Although not statistically significant, a comparison between presoldier formation after phase one (Figure 6-1A) and after phase two (Figure 6-1B) suggested that after a lag time, presoldiers can be formed while feeding on untreated cardboard if allowed to feed on pyriproxyfen treated cardboard for at least two weeks prior. However, 12.5% presoldier induction would probably not adversely affect the termite cohort. Under these laboratory conditions, cohort demise would be expected if soldier proportions increased to ≥20% for a period of about 4 wk (see Chapter 5).

Termites which fed for 4 wk continuously on cardboard with 100 or 150 ppm pyriproxyfen produced significantly higher numbers of presoldiers than did termites which fed on cardboard with 0 or 50 ppm pyriproxyfen. Termites which fed on cardboard treated with 50 ppm produced significantly higher numbers of presoldiers than did termites which fed on cardboard treated with 0 ppm (Figure 6-1C). After phase two, termites which previously fed on cardboard with 100 ppm pyriproxyfen still retained a significantly higher number of presoldiers and soldiers than did termites which fed on 0, 50, and 150 ppm pyriproxyfen, but actual numbers were reduced due to mortality (Figure 6-1D). After phase two, termites which previously fed on cardboard treated with 50 and 150 ppm also retained significantly higher numbers of presoldiers and soldiers than did termites which fed on cardboard treated with 0 ppm (Figure 6-1D).

Examination of total mortality after phase two indicated that termite cohorts which fed continuously on 150 ppm pyriproxyfen for 2 or 4 wk tended to have the highest mortality, although not statistically significant (Table 6-2). Mortality may have been induced by the production of soldier proportions greater than the termite cohort could support (see Chapter 5). Mortality may also be due to other factors which were not investigated in this project since termites which were allowed to feed on cardboard treated with acetone-only consistently resulted in lower survival after phase one and two, although not always statistically significant (Table 6-2).

Intermittent feeding on treated and then untreated cardboard did not produce significant numbers of presoldiers and soldiers during phase two ($p<0.0589$). Visual inspection of termites while transferring treated and untreated cardboard rolls during phase one also indicated that there was no significant presoldier or soldier formation in this part of the assay. In addition to not consuming quantities of pyriproxyfen need for presoldier formation, lack of presoldier formation could be due to the bioassay technique which resulted in high mortality and the elimination of over half of the EUs (Table 6-2).

There was no significant difference in cardboard weight loss for any combination of time interval, phase and ppm, except on the 0 ppm cardboard rolls during intermittent feeding (Table 6-3). Significant cardboard weight loss at this level can be attributed to the high consumption of one colony. Increased replication would probably result in loss of significance.

The presoldier and soldier formation in this study with R. virginicus greatly exceeded the that of Su & Scheffrahn (1989b). At 4 wk of feeding in a no-choice bioassay at 150 ppm, 26.2±7.7% of the R. flavipes cohort became soldiers or presoldiers, with 10.0±3.3 presoldiers (Su & Scheffrahn 1989b). In this study with R. virginicus, virtually no soldiers were produced, but 52.4% presoldiers were formed. Presoldiers formed after feeding on pyriproxyfen differed in morphology from naturally occurring presoldiers, appearing to have a smaller head capsule which melanized slightly and smaller mandible formations, than naturally occurring presoldiers.

Worker reduction was defined as the number of dead workers, as well as those which molted into presoldiers and soldiers (Su & Scheffrahn 1989b). Worker reduction due to pyriproxyfen could not be assessed due to high mortality in acetone-only treated cardboard. However, Su & Scheffrahn (1989b) found that after 4 wk, at 150 ppm, in a no-choice bioassay, worker reduction was 61.7±5.5%, indicating significant potential of this active ingredient toward controlling R. flavipes.

The effect of intermittent feeding in the laboratory under these circumstances does not remotely represent conditions in the field. A better bioassay may be to impregnate a less preferred, but still acceptable food source such as filter paper, and place both treated filter paper and untreated cardboard in the paired design (see Chapter 4). Results of Chapter 4 indicated that termites can be generalist cellulose feeders and would probably feed on both substrates even if cardboard is preferred. This bioassay design allows termites to feed freely between to two food sources, thus termites would be feeding intermittently on the treated substrate without disruptions by the experimenter which inevitably cause mortality. Results of this bioassay could be compared against one where both food types could be treated at a non-repellent concentration.

In summary, results from this study suggest that continuous feeding on pyriproxyfen for at least 4 wk at 100 or 150 ppm is required to reach the threshold of ≥20% presoldier and soldier formation which can cause cohort

demise in the laboratory. This requirement may be a disadvantage for pyriproxyfen because factors which affect patch choice and food selection are not well defined for Reticulitermes spp. Feeding time or dose required to effect control with other IGRs such as hexflumuron, a CSI, are unknown.

Table 6-1. Paired t-test comparing numbers of R. virginicus soldiers and presoldiers after continuous feeding on treated cardboard for two or four weeks and after feeding on untreated cardboard for four weeks

Time Interval	PPM	Prob>t	N
2C[1]	0	0.1817	4
	50	0.0357	5
	100	0.8448	5
	150	0.4226	3
4C[2]	0	0.0756	6
	50	0.1791	6
	100	0.6832	7
	150	0.1452	7

[1] Continuous feeding for two weeks
[2] Continuous feeding for four weeks

Table 6-2. Total mortality of R. virginicus after phase one and phase two for each time interval and concentration of pyriproxyfen tested

Time Interval	PPM	Prob>F[1]	Mortality[1]	N[1]	Prob>F[2]	Mortality[2]	N[2]
2C	0	0.0140	28.6±15.0a	8	0.0154	53.6±20.8a	9
	50		27.8±19.4a	14		44.1±22.0a	8
	100		21.0±15.1a	13		49.3±11.7a	9
	150		29.6±18.4a	13		53.9±18.9a	7
4C	0	0.0206	29.8±11.9a	13	0.0232	67.5±14.1b	6
	50		28.9±18.8a	18		49.5±10.4a	6
	100		20.6±10.0a	14		56.4±13.6ab	7
	150		32.1±12.0a	17		67.9±14.0b	7
4I	0	.	.	0	0.0110	71.4±14.2a	5
	50		.	0		72.3±17.4a	6
	100		.	0		63.3±17.1a	8
	150		.	0		62.8±25.1a	6

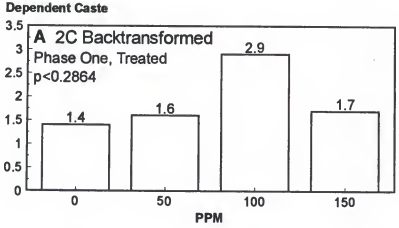
[1] Statistics of phase one
[2] Statistics of phase two

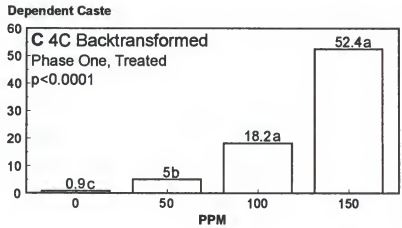
Table 6-3. Cardboard weight loss due to feeding by R. virginicus, for each time interval of continuous and intermittent feeding, and concentration of pyriproxyfen for phases one and of sample size N

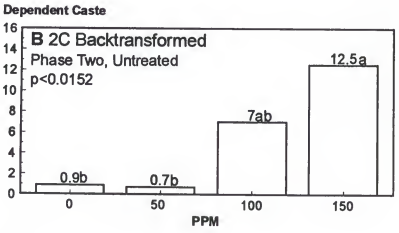
Time Interval	PPM	Prob>F[1]	Mean CBWL (mg)[1,3]	N[1]	Prob>F[2]	Mean CBWL (mg)[2,3]	N[2]
2C	0	0.0292	77.1±60.5a	12	0.0001	139.1±102.5a	9
	50		105.1±44.3a	18		170.1±83.9a	10
	100		100.0±38.8a	17		142.1±46.7a	10
	150		77.9±47.3a	18		103.1±17.0a	7
4C	0	0.0001	161.7±22.1a	13	0.1033	131.2±47.8	6
	50		168.5±46.9a	17		114.3±46.2	6
	100		154.4±24.6a	13		209.2±133.3	8
	150		152.9±43.6a	17		158.1±35.9	10
4I	0	0.0001	69.8±63.0b	13	0.3534	319.4±42.6	5
	50		26.9±5.0a	16		319.7±154.7	6
	100		32.0±13.7a	15		303.2±77.4	8
	150		28.8±7.5a	14		316.2±85.0	6

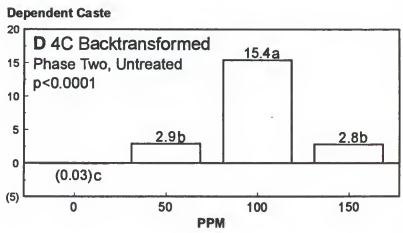
[1] Statistics of phase one
[2] Statistics of phase two
[3] Cardboard weight loss

Figure 6-1. Backtransformed presoldier and soldier formation after feeding continuously on pyriproxyfen at 0, 50, 100, and 150 ppm for two (A) or four weeks (C) during phase one. Backtransformed presoldier and soldier formation after feeding four weeks on untreated cardboard in phase two for R. virginicus which previously fed continuously for two (B) and four (D) weeks on pyriproxyfen.


Dependent Caste
A 2C Backtransformed
Phase One, Treated
p<0.2864
1.4
1.6
2.9
1.7
PPM


Dependent Caste
C 4C Backtransformed
Phase One, Treated
p<0.0001
0.9c
5b
18.2a
52.4a
PPM


Dependent Caste
B 2C Backtransformed
Phase Two, Untreated
p<0.0152
0.9b
0.7b
7ab
12.5a
PPM


Dependent Caste
D 4C Backtransformed
Phase Two, Untreated
p<0.0001
(0.03)c
2.9b
15.4a
2.8b
PPM

CHAPTER 7
FIELD EVALUATION OF PYRIPROXYFEN ON R. VIRGINICUS IN GAINESVILLE, FLORIDA

Introduction

The juvenile hormone mimic, pyriproxyfen, has been evaluated for control of the Formosan (Haverty et al. 1989, Su & Scheffrahn 1989b) and eastern subterranean termites (Su & Scheffrahn 1989b). Su & Scheffrahn (1989b) found that after 4 wk of continuous exposure in a choice bioassay, the soldier and presoldier proportion increased to 39.2% with a worker reduction of 45.9% for R. flavipes. In Chapter 6, presoldier intercaste proportions did not increase to levels high enough to cause cohort demise (see Chapter 5) after treated food sources were removed for intermittent feeding or continuous feeding for 2 wk. Thus, one potential disadvantage to pyriproxyfen is the apparent necessity of continuous feeding on the bait in order to gain control.

Su (1994), however, has shown that subterranean termites can consume sufficient quantities of the chitin synthesis inhibitor, hexaflumuron (DowElanco, Indianapolis, Indiana), to gain suppression and elimination of the colony. The purpose of this study was to evaluate the efficacy of pyriproxyfen in controlling R. virginicus in two field sites.

Materials and Methods

Trial 1. A field colony at Austin Cary Memorial Forest was selected for the first field trial of pyriproxyfen, in August 1992. Colony size was determined using the triple-mark-recapture method and Nile Blue A as a dye marker (Su & Scheffrahn 1988b, see Chapter 2). Worker weights and soldier proportions were recorded for comparison after treatment of the colony (Appendix B).

Pine board (Pinus spp.) were oven dried at 80°C for at least 48 h and cooled in a desiccator. Pine boards were vacuum impregnated using methods identical to those of Su et al. (1991b) with acetone only or pyriproxyfen dissolved in acetone at approximately 162 ppm (wt A. I./ wt wood). This concentration was slightly higher than the 30-150 ppm determined to be non-repellent and effective in causing significant pre-soldier formation for R. flavipes (Su & Scheffrahn 1989b). Three acetone treated boards and three boards impregnated with pyriproxyfen were nailed together to comprise the bait block These baits were placed in underground monitoring stations (Su & Scheffrahn 1986) of the field colonies. After one month, these traps were checked for termite activity.

Trial 2. An aerial infestation in the fourth floor of Yon Hall, University of Florida, Gainesville, was identified in late May 1993 in room 420. Yon Hall, an athletic dormitory built in the 1960's, is a steel and concrete four-story structure with 112 units, surrounded by an asphalt parking lot. At the end of the 1993 spring semester, a student discovered the infestation while moving out when he picked up a stack of termite infested books on the infested wooden shelf. The

room had been treated with conventional termite control methods within the prior 18 months for an active infestation (Ken Glover, UF Pest Control, personal communication). Inspection of the room revealed water marks on the ceiling and wall where the closet was located. Inspection of the roof also revealed a moisture problem, because the cellulosic roofing insulation was spongy in areas.

Infestation was heaviest under the floor tiles in the corner of the closet. On June 3, 1993, moistened cardboard rolls were placed where the infestation was the heaviest. Cardboard pieces were held in place by wooden blocks and covered with plastic to retain moisture. The mark-recapture process was started on June 8, 1993.

Once the colony was characterized, cardboard rolls (3 cm diam, 8 cm high) were treated with pyriproxyfen as described in Chapter 6, so final concentrations were 0, 50, 75, 100, and 150 ppm (wt A. I./wt cardboard). Cardboard rolls were arranged in a randomized complete block design with five blocks. These rolls were taped together in a square, moistened with tap water, and placed in the corner with the heaviest infestation. The rolls were again covered with plastic to retain moisture. Factors controlling termite food choice were unknown, thus, this design served a choice test for this situation. Termites were allowed to feed for 3 wk.

Results

Trial 1. Colony size was determined to be over 2 million R. virginicus, covering an area of approximately 36 m^2 (see Chapter 2). After one month,

there was no termite activity in any of the traps containing the vacuum impregnated boards. None of the traps appeared to have sustained any feeding damage.

Termites may have vacated traps because the 162 ppm was repellent to the field colony of R. virginicus. Furthermore, the impregnation procedure may not have resulted in uniform distribution of the toxicant, causing the concentration at the surface of the wood block to be higher after evaporation (Smith 1961). However, not even control panels were infested, so the impregnation procedure may have caused the wood to become unpalatable to R. virginicus, especially in the presence of alternate food sources, by increasing wood rigidity. These wood panels were significantly more brittle when nailed together. The colony at Austin Cary Memorial Forest had an abundance of alternate food sources which could have provided harborage for the termites. In fact, Nile Blue A stained termites were found in fallen branches and other wood debris on the soil surface. Thus, the colony was still in the vicinity of the recorded foraging area.

Trial 2. Termites from the colony at Yon Hall were also identified as R. virginicus. The population was estimated to contain 148,995±2,884 individuals. Mean worker weight was 2.16±0.04 mg; mean soldier weight was 2.68±0.06 mg. Only two cycles of mark-recapture were completed (Table 7-1) because standard errors were low (1.94%).

Treated cardboard was placed on July 22, 1993. No termite activity was detected after about 3 wk of access to the treated cardboard rolls, although there were signs of feeding. Three possible explanations exist for the absence of termite activity. First, termites found the treated cardboard unpalatable and moved, possibly into the cellulosic roofing material. Second, the colony was suppressed. Third, the colony was eliminated. There were no other monitoring stations available to confirm whether the termites were simply repelled from the area because of the pyriproxyfen-treated cardboard. If termites were still actively foraging, termites were assumed to again find untreated cardboard an acceptable food source. Untreated cardboard rolls were left in place for about one month, but no infestation reappeared. After two months of termite inactivity, the room had to be prepared for incoming students, thus follow up was done through UF Pest Control. After almost one year, termites have not returned to room 420 or any part of this building.

Discussion

Clearly, R. virginicus was not controlled at the Austin Cary site. The impregnation procedure did not affect R. flavipes in feeding on treated and untreated wooden blocks in a choice test at a concentration close to what was used in this study (Su & Scheffrahn 1989b). However, the termites were confined to a jar, limiting food to blocks which both underwent an impregnation procedure. Furthermore, I have observed that R. virginicus tended to be found in wood which was previously excavated by R. flavipes (see Chapter 2). During

the program, R. virginicus have been almost exclusively collected from logs which were more decayed than the solid wood of logs, where R. flavipes have been found. These observations suggest that R. virginicus prefers less solid food types than R. flavipes and given a choice in a field situation, R. virginicus appeared to prefer wood which had not undergone the impregnation procedure.

R. virginicus was possibly controlled at the Yon Hall site. There was no way to known if termites relocated, except to wait for another infestation to appear. However, colony suppression or elimination was possible based on the following deduction. Soldier proportion studies (see Chapter 4) indicated that cohorts which must support ≥20% soldiers can die in about 4 wk. Su & Scheffrahn (1989b) have shown in a choice test that 39.2% soldiers and presoldiers can form approximately 4 wk after feeding on pyriproxyfen impregnated wood cubes at 150 ppm. In Chapter 5, cardboard was shown to be preferred over filter paper, a relatively pure cellulose source. Thus, the possibility exists that R. virginicus fed on the treated cardboard rolls, rather than the cellulosic roofing material, long enough to receive a dose which caused soldier proportions to increase ≥20%. Colony demise followed by 4 wk.

In order to maintain continuous feeding, termites must remain with the bait station or return regularly to it. Establishing bait stations has been difficult in Gainesville, Florida, (see Chapter 2) because factors for "patch choice" are undefined. Even in urban southeastern Florida, only about 5-10% of the wooden stakes placed next to an infested structure were attacked after 5 years (Su

1991). However, once subterranean termites discover a food source, feeding strategies appear to differ C. formosanus and R. flavipes. Formosan subterranean termites appear to forage more intensely, consuming a greater percentage of one food source before moving on to an equivalent food source, while R. flavipes tends to feed less intensely at individual food sources, consuming similar amounts of food at visited food sources (Delaplane & La Fage 1989c). The ability of termites to chose a food source in a field situation, and especially in the case of Reticulitermes spp., feed transiently, can present an obstacle for field testing pyriproxyfen for termite baits, where continuous feeding for periods ≥4 wk appears to be required.

Table 7-1. Number of total R. virginicus captured (n_i), marked captured (m_i), and marked released (r_i) for the aerial infestation in Yon Hall.

	Time		
	1	2	3
Total Captured (n_i)	---	11,101	2,747
Marked Captured (m_i)	---	1,922	746
Marked Released (r_i)	36,342	10,727	---

CHAPTER 8
CONCLUSION

The purpose of this study was to provide information on the biology and behavior of subterranean termites so that reduced chemical tactics, such as baiting, can be efficiently employed. One objective of this study (see Chapter 2) was to evaluate the foraging dynamics of Reticulitermes spp. in wooded areas of Gainesville, Florida, since evidence from other termite species suggests that populations and territories differ depending on habitat (Lee & Wood 1971). This study provided background information on colonies used to assess pyriproxyfen in baits to control R. virginicus. Colonies in wooded areas of Gainesville, Florida, were typically smaller (17,000 to 2 million individuals) than colonies in developed areas (Esenther 1980a, Grace et al. 1989, Grace 1990, Su et al. 1993). In addition, several colonies were found in a given study site, often moving into a territory previously occupied by a different species of subterranean termite. An attempt to identify long-term dye markers for Reticulitermes spp. so that multiple colonies in a given field site could be studied simultaneously, failed (see Chapter 3). Currently, only Nile Blue A (Su et al. 1991a) appears to be suitable as a long-term marker for Reticulitermes spp.

A correlation analysis of foraging distance (m) and population size on data from this and previous foraging studies suggested that food and haborage may be important factors limiting colony expansion in Reticulitermes spp. A laboratory study (see Chapter 4) on the effect of food placement and food type on feeding preference indicated that there was a propensity for termites to feed at a single site even if food types were equivalent under the described conditions. Thus, termites may search for food randomly, but feeding was not random under these laboratory conditions. Non-random feeding may be due to recruitment to an acceptable food source.

The last half of this project focused on the feasibility of using pyriproxyfen as an active ingredient for baits. Pyriproxyfen is a Juvenile Hormone Mimic (JHM) which causes superfluous soldier intercaste production. If there are more soldiers in a colony than workers can support, the colony will die.

A laboratory study (see Chapter 5) indicated that cohorts of R. virginicus workers could not support soldiers in excess of 20% for longer than 4 wk. Soldiers in excess of 20% were produced when termite cohorts were allowed continuous access to pyriproxyfen impregnated rolls for 4 wk; however, intermittent feeding or 2 wk of continuous feeding on pyriproxyfen-treated cardboard did not result in 20% presoldier formation, in the laboratory (see Chapter 6). Results for field trials of pyriproxyfen to control R. virginicus were equivocal (see Chapter 7). Termites were not controlled at the Austin Cary field site, possibly because of the abundance of alternate food sources. The colony

at Yon Hall may have been controlled because the colony appeared to be food limited and probably chose to feed on cardboard, a preferred food source (see Chapter 4), at least compared to filter paper.

In conclusion, foraging dynamics appear to be habitat dependent. The presence or absence of alternate food sources in the habitat appears not only to drive the expansion or limitation of foraging distance in Reticulitermes spp., but also wooden bait block acceptance. Once an acceptable food source is located, recruitment is probably an important factor in food choice for Reticulitermes spp. In this study, underground bait stations have been difficult to establish in wooded areas of Gainesville, Florida, probably because copious amounts of alternate food exist for Reticulitermes spp. The ability of termites to chose a food source in a field situation, can present an obstacle for field testing pyriproxyfen for termite baits, where continuous feeding for long periods seems to be a requirement. Baits with an active ingredient like pyriproxyfen will probably have to be comprised of a food source that is preferred over the alternate food sources which are found in the field so that Reticulitermes spp. can first locate, then choose the bait as a food source before recruiting others.

APPENDIX A. Percentage of marked Reticulitermes spp. workers recovered from each trap for each recapture interval of the various field sites where n_i is the total number captured or recaptured, m_i is the number marked, and r_i is the total number released. χ^2-values were calculated using the variance to mean ratio. If $\sigma^2=\mu$, then the distribution was random; if $\sigma^2<\mu$, then distribution was uniform; if $\sigma^2>\mu$,, the distribution was contagious

Colony	Recapture	Trap	n_i	m_i	r_i	% m_i/n_i	Ave m/n (±SEM)	χ^2-Value	Distribution Type
ANSCI IA	0	1	---	---	3,396	---			
	1	1	3,254	185	3,109	5.68	6.25±3.16	4.79	Random
		4	1,115	116	1,048	10.40			
		5	1,276	79	1,080	6.19			
		10	3,825	104	2,934	2.72			
	2	1	---	---	---	---	23.45±5.12	2.24	Random
		4	341	77	334	22.58			
		5	304	88	298	28.95			
		10	4,101	815	3,684	18.81			
	3	1	---	---	---	---	18.17±4.07	0.91	Random
		4	---	---	---	---			
		5	1,601	337	---	21.05			
		10	1,694	259	---	15.29			
ANSCI IB	0	1	1,495	---	---	---	0.50±0.45	0.81	Random
	1	1	2,512	24	---	0.96			
		4	4,177	20	---	0.48			
		13	6,668	4	---	0.06			

APPENDIX A (cont.)

Colony	Recapture	Trap	n_i	m_i	r_i	% m_i/n_i	Ave m/n (±SEM)	χ^2-Value	Distribution Type[1]
	2	1	---	---	---	---	---		
		4	---	---	---	---			
		13	---	---	---	---			
	3	1	---	---	---	---	---		
		4	---	---	---	---			
		13	---	---	---	---			
ANSCI II	0	1	5,307	---	---	---	---		
	1	1	4,051	49	3,628	1.21	0.66±0.77	0.90	Random
		3	2,602	3	2,315	0.12			
	2	1	1,941	40	1,636	2.06	2.17±1.22	2.06	Random
		3	5,173	178	3,181	3.44			
		7	7,776	257	6,089	3.31			
		8	893	9	727	1.01			
		1	1,526	237	---	15.53	19.23±13.07	17.75	Contagious
		3	---	---	---	---			
		7	2,388	806	---	33.75			
		8	3,054	257	---	8.42			
HU	0	8	2,137	---	---	---	---		
	1	6	6,081	386	5,252	6.35	8.29±2.74	0.91	Random
		8	352	36	0	10.23			

APPENDIX A (cont.)

Colony	Recapture	Trap	n_i	m_i	r_i	% m_i/n_i	Ave m/n (±SEM)	χ^2-Value	Distribution Type[1]
	2	6	1,130	259	985		22.92±.		
		8	---	---	---				
	3	6	1,180	526	---		44.58±.		
		8	---	---	---				
AC2	0	16	803	---	---	---	---		
	1	16	541	25	459	4.62	4.62±.		
	2	16	258	34	---	13.18	13.18±.		
AC3	0	16	16,914	---	---	---	---		
	1	12	5,657	157	4,676	2.78	1.75±0.96	1.05	Random
		7/21	10,679	168	15,380	1.57			
		20	29,296	240	23,924	0.89			
	2	12	7,225	50	7,024	0.69	7.82±8.53	46.52	Contagious
		13	1,488	12	1,278	0.81			
		16	2,038	88	2,033	4.32			
		17	801	111	721	13.86			
		21	135	30	74	22.22			
		22	1,638	82	1,117	5.01			
	3	16	523	48	---	9.18	4.34±2.27	7.12	Random
		22	1,096	36	---	3.28			
		21	1,756	74	---	4.21			

APPENDIX A (cont.)

Colony	Recapture	Trap	n_i	m_i	r_i	% m_i/n_i	Ave m/n (±SEM)	χ^2-Value	Distribution Type[1]
		14	842	36	---	4.28			
		12	4,922	200	---	4.06			
		20	14,430	301	---	2.09			
		40	2,028	66	---	3.25			
YON	0	1	36,342	---	---	---	---		
HALL	1	1	10,727	1,922	11,101	17.92	17.92±.		
	2	1	2,747	746	---	27.16	27.16±.		

APPENDIX B. Summary table of the numbers, weights (± 0.01 mg), and soldier proportion for termites from all castes of Reticulitermes spp. for each field site used in this study.

CLNY[1]	DATE[2]	T[3]	S/N S[4]	W[5]	W_WT[6]	S[7]	S_WT[8]	PS[9]	PS_WT[10]	N[11]	N_WT[12]	S[13]
AC[14]	5/31/91	2	NS	10,440	1.45±0.04	161	2.19±0.06	80	---[17]	1	---	1.52
	6/16/91	26	NS	177	1.51±0.05	22	2.22±0.08	---	---	---	---	11.00
	3/17/92	11	NS	939	1.50±0.08	24	2.05±0.03	---	---	---	---	2.49
		16	S	940	1.52±0.07	19	2.02±0.05	---	---	---	---	1.98
	4/3/92	16	S	541	1.54±0.02	4	1.98±---	---	---	---	---	0.70
	4/16/92	16	S	258	1.41±0.04	0	0	---	---	---	---	0.00
		13	NS	12,536	1.67±0.04	124	2.54±0.10	---	---	---	---	1.01
	4/23/92	13	NS	8,964	1.65±0.09	0	0	---	---	---	---	0.00
	5/27/92	13	NS	20,995	1.87±0.04	800	2.57±0.08	---	---	---	---	3.67
		15	NS	3,509	1.51±0.06	10	2.12±---	---	---	---	---	0.28
		16	NS	1,907	1.56±0.06	13	2.94±---	---	---	---	---	0.68
		10	NS	911	1.45±0.22	10	1.84±---	---	---	---	---	1.09
		5	NS	---	1.61±0.18	12	2.27±---	---	---	---	---	---
		21	NS	---	1.96±0.06	135	2.53±0.14	---	---	---	---	---
	7/15/92	16	S	16,914	1.85±0.07	---	---					---
		13		19,864	1.91±0.04	131	2.59±0.04	---	---	---	---	0.66

APPENDIX B (con.t)

CLNY[1]	DATE[2]	T[3]	S/N S[4]	W[5]	W_WT[6]	S[7]	S_WT[8]	PS[9]	PS_WT[10]	N[11]	N_WT[12]	S[13]
AC[14]		20		12,835	1.88±0.10	79	2.63±0.08	---	---	---	---	0.27
		22		8,992	1.82±0.08	101	2.73±0.18	62	2.59±0.10	---	---	1.11
		25		30	1.79±0.04	0	0	---	---	---	---	0.00
		36		7,788	1.47±0.03	12	3.28±---	---	---	---	---	0.15
		5		1,207	1.58±0.04	9	3.21±---	---	---	---	---	0.70
		6		684	2.02±0.27	6	2.43±---	---	---	---	---	0.87
		9		57	2.10±0.10	0	0	---	---	---	---	0.00
		11		61	1.81±0.10	3	2.27±---	---	---	---	---	4.69
	7/30/92	5		1,491	1.58±0.04	90	2.06±0.06	51	2.02±0.04	938	2.52±0.05	5.69
		12		5,657	1.85±0.05	198	2.43±0.07	---	---	---	---	3.38
		17		3,013	2.01±0.37	95	2.46±0.07	46	2.38±0.09	---	---	3.06
		20		29,536	1.74±0.07	293	2.54±0.07	---	---	---	---	0.98
		21		7,834	1.77±0.08	135	2.39±0.09	---	---	---	---	1.69
	8/14/92	5	NS	713	1.45±0.11	16	2.15±0.22	---	---	---	---	2.19
		6	NS	387	1.54±0.08	12	2.21±0.05	---	---	---	---	3.01
		25	NS	1,411	1.81±0.09	61	2.35±0.03	---	---	---	---	4.14
		31	NS	975	1.46±0.05	6	2.07±---	4	---	---	---	0.61
		36	NS	1,066	1.55±0.08	25	2.31±0.07	4	---	---	---	2.29
		12	S	7,307	1.75±0.08	232	2.48±0.11	---	---	---	---	3.08
		13	S	1,514	1.84±0.10	10	2.64±---	---	---	---	---	0.66
		14	S	2,138	1.85±0.09	51	2.41±0.05	---	---	---	---	2.33
		17	S	902	1.87±0.23	27	2.47±0.05	---	---	---	---	2.91
		21	S	165	2.16±0.20	2	2.50±---	---	---	---	---	1.20
		22	S	1,720	1.81±0.13	14	2.44±0.09	---	---	---	---	0.81

APPENDIX B (con.t)

CLNY[1]	DATE[2]	T[3]	S/N S[4]	W[5]	W_WT[6]	S[7]	S_WT[6]	PS[9]	PS_WT[10]	N[11]	N_WT[12]	S[13]
AC[14]	8/27/92	40	S	2,094	1.91±0.13	47	2.63±0.14	---	---	---	---	2.20
		16	S	571	1.84±0.04	14	2.52±0.03	---	---	---	---	2.39
		22	S	1,132	1.82±0.12	31	2.38±0.06	---	---	---	---	2.67
		21	S	1,830	1.66±0.04	53	2.47±0.07	---	---	---	---	2.81
		14	S	878	1.87±0.09	18	2.45±0.04	---	---	---	---	2.01
		12	S	5,166	1.83±0.16	121	2.49±0.07	---	---	---	---	2.29
		20	S	14,731	1.69±0.14	276	2.59±0.11	---	---	---	---	1.84
	9/1/92	36	NS	620	1.42±0.07	17	2.23±0.09	---	---	---	---	2.67
		6	NS	120	1.42±0.05	4	2.18±---	---	---	---	---	3.23
		31	NS	919	1.49±0.11	6	2.48±---	---	---	---	---	0.65
		25	NS	3,078	1.82±0.11	145	2.46±0.10	---	---	---	---	4.50
		2	NS	86	0.97±0.03	4	1.15±---	---	---	---	---	4.44
	9/3/92	14	S	720	1.90±0.08	6	2.60±---	---	---	---	---	0.83
		21	S	1486	1.78±0.08	29	2.35±0.06	---	---	---	---	1.91
		20	S	11,915	1.69±0.13	214	2.41±0.11	---	---	---	---	1.76
		12	S	4508	1.81±0.08	35	2.42±0.05	---	---	---	---	0.77
ANSCI[15]	5/08/91	1	S	5,248	2.63±0.05	15	3.89±0.13	112	---	---	---	2.36
	5/28/91	1	S	3,254	2.91±0.13	30	4.07±0.20	1	---	---	---	0.94
		4	S	1,115	2.93±0.09	15	4.05±---	4	---	---	---	1.68
		12	NS	3,678	2.24±0.19	102	3.47±0.07	25	---	---	---	3.34
		5	S	1,276	2.74±0.10	13	4.05±---	48	---	---	---	4.56
		10	S	3,825	2.67±0.17	50	4.20±0.19	182	---	---	---	5.72
		13	NS	246	2.95±0.08	3	2.94±0.05	2	---	---	---	1.99

APPENDIX B (con.t)

CLNY[1]	DATE[2]	T[3]	S/N S[4]	W[5]	W_WT[6]	S[7]	S_WT[8]	PS[9]	PS_WT[10]	N[11]	N_WT[12]	S[13]
ANSCI I[15]	6/10/91	1	NS	2,117	2.25±0.04	26	2.85±0.08	0	---	---	---	1.21
		4	S	341	2.70±0.06	3	2.65±---	0	---	---	---	0.87
		5	S	304	2.60±0.09	0	0	0	---	---	---	0
		10	S	3,456	2.76±0.09	16	4.15±0.28	0	---	---	---	0.46
		13	NS	532	3.29±0.10	18	4.90±0.03	0	---	---	---	3.27
	6/29/91	1	S	2,512	2.14±0.04	30	2.99±0.08	0	---	---	---	1.18
		4	S	4,177	2.11±0.05	28	2.91±0.04	0	---	---	---	0.67
		5	S	1,601	2.30±0.13	13	4.36±0.04	0	---	---	---	0.81
		10	S	1,953	2.40±0.20	14	4.53±0.02	0	---	---	---	0.71
		13	S	6,664	2.010.04	10	3.21±0.02	0	---	---	---	0.15
		8	NS	1,093	2.10±0.17	41	3.56±0.09	0	---	---	---	3.62
	9/03/91	10	---	3,656	2.43±0.17	29	3.95±0.05	0	---	---	---	0.79
	11/07/91	13	---	1,729	1.97±0.06	24	2.64±0.02	0	---	---	---	1.37
		10	---	552	2.70±0.16	88	4.30±0.05	0	---	---	---	13.75
		11	---	4,256	1.93±0.08	542	2.63±0.08	0	---	---	---	11.30
	4/29/92	10	---	223	3.15±0.11	0	---	---	---	---	---	
ANSCI II[16]	9/09/91	1	S	10,422	2.25±.	300	4.11±0.08	48	3.37±0.12	81	3.89±0.09	3.00
	9/23/91	1	S	4,071	2.41±0.10	81	4.22±0.17	40	3.37±0.13	88	4.04±0.07	2.83
		3	S	2,602	2.55±0.12	16	3.68±0.05	0	0	15	3.17±0.17	0.58
		4	NS	8,253	2.52±0.10	328	3.36±0.14	41	0	53	3.35±0.22	4.25
		6	NS	1,105	2.32±0.19	36	4.29±0.07	3	0	26	3.29±0.27	3.33

APPENDIX B (con.t)

CLNY[1]	DATE[2]	T[3]	S/N S[4]	W[5]	W_WT[6]	S[7]	S_WT[8]	PS[9]	PS_WT[10]	N[11]	N_WT[12]	S[13]
ANSCI II[16]	10/10/91	1	S	1,941	2.24±0.10	13	4.10±0.06	0	0	0	0	0.67
		13	S	5,173	2.62±0.12	59	3.70±0.08	5	0	43	3.48±0.20	1.13
		6	NS	1,051	2.47±0.07	53	4.17±0.09	0	0	0	0	4.80
		7	S	7,776	2.58±0.13	94	3.71±0.09	2	0	88	3.51±0.13	1.10
		8	S	893	2.25±0.11	14	3.53±0.08	0	0	24	3.20±0.26	1.50
		9	NS	462	2.77±0.11	11	4.19±0.02	0	—	0	—	2.33
		10	NS	720	2.67±0.13	12	4.20±0.03	0	0	0	—	1.64
	10/23/91	1	S	1,526	2.48±0.06	20	4.04±0.07	0	0	0	—	1.29
		3	NS	4,768	1.98±0.08	98	2.54±0.16	0	0	0	—	2.01
		7	S	2,388	2.58±0.12	14	3.49±0.04	0	0	44	3.56±0.39	0.57
		8	S	3,053	2.76±0.07	15	3.94±0.15	0	0	44	4.46±0.33	0.48
	31292	1	S	3,679	2.62±0.14	189	3.90±0.13	0	0	0	0	4.89
		6	NS	406	3.02±0.06	3	4.00±—	0	0	0	0	0.73
		7	S	4,721	2.60±0.15	65	3.69±0.09	0	—	0	—	1.36
		8	S	6,828	2.67±0.12	—	3.73±0.06	0	0	0	0	—
		9	NS	1090	2.95±0.14	17	4.05±0.16	0	0	0	0	1.54
		10	NS	164	3.09±0.05	12	4.26±0.02	0	0	0	0	6.82
	50592	6	NS	1,706	2.75±0.13	5	4.26±—	0	0	0	0	0.29
		8	NS	3,973	2.18±0.17	24	3.61±—	0	0	0	0	0.60
		9	NS	335	1.93±0.07	8	2.81±—	0	0	0	0	2.33
	7/13/92	12	NS	6,046	2.68±0.15	8	3.44±—	0	0	0	0	0.13
		6	NS	8,213	2.79±0.09	46	4.14±0.10	6	0	0	0	0.63
	8/12/92	6	NS	4,054	2.87±0.15	50	4.19±0.11	—	—	—	—	1.22

APPENDIX B (con.t)

CLNY[1]	DATE[2]	T[3]	S/N S[4]	W[5]	W_WT[6]	S[7]	S_WT[8]	PS[9]	PS_WT[10]	N[11]	N_WT[12]	S[13]
		11	NS	5,264	2.06±0.13	216	2.71±0.08	—	—	—	—	3.94
		12	NS	7,800	2.31±0.23	93	3.43±0.05	30	0	0	0	1.55
HU	9/03/91	8	S	2,922	2.06±0.13	77	2.99±0.07	0	0	0	0	2.57
	9/16/91	2	NS	6,692	2.27±0.07	96	3.29±0.08	0	0	0	0	1.41
		3	NS	6,341	2.27±0.11	159	3.56±0.07	0	0	0	0	2.45
		5	NS	6,060	2.24±0.05	88	3.28±0.07	0	0	0	0	1.43
		6	S	6,031	2.18±0.16	117	3.15±0.09	12	0	0	0	2.09
		8	S	352	—	18	—	0	0	0	0	4.86
		10	NS	5,788	2.45±0.05	94	3.67±0.03	0	0	0	0	1.60
		16	NS	300	2.35±0.14	2	—	0	0	0	0	0.66
	10/01/91	2	NS	1,413	2.36±0.06	32	3.25±0.08	0	0	1	—	2.21
		3	NS	21	2.23±0.11	0	0	0	0	0	0	0
		6	S	1,130	2.00±0.02	24	3.21±0.16	0	0	1	—	2.08
		9	NS	258	1.73±0.02	6	2.68±—	0	0	0	0	2.27
		10	NS	646	2.49±0.10	7	3.93±—	0	0	0	0	1.07
		11	NS	271	1.50±0.03	6	2.80±—	0	0	0	0	2.17
	10/03/91	11	NS	8,616	1.88±0.06	250	2.46±0.05	0	0	0	0	2.82
	10/16/91	2	NS	312	2.31±0.16	7	3.24±—	0	0	0	0	2.19
		5	.	349	2.37±0.13	3	3.33±—	0	0	0	0	0.85
		6	S	1,180	1.93±0.12	13	3.22±0.02	0	0	0	0	1.09
		9	NS	125	1.79±0.04	4	2.78±—	0	0	0	0	3.10
		10	NS	2,002	2.45±0.12	21	3.71±0.07	0	0	0	0	1.04
		11	NS	285	1.53±0.18	1	2.70±—	0	0	0	0	0.35

APPENDIX B (con.t)

CLNY[1]	DATE[2]	T[3]	S/N S[4]	W[5]	W_WT[6]	S[7]	S_WT[8]	PS[9]	PS_WT[10]	N[11]	N_WT[12]	S[13]
		12	NS	352	1.80±0.05	15	2.52±0.06	0	0	0	0	4.09
		13	NS	3,240	1.92±0.06	---	2.68±0.05	0	0	0	0	---
HU		14	NS	360	1.53±0.03	5	3.00±---	0	0	0	0	1.37
		15	NS	178	1.50±0.04	3	2.67±---	0	0	0	0	1.66
	11/07/91	8	NS	1,548	2.28±0.12	20	3.74±0.02	0	0	0	0	1.28
		2	NS	598	2.24±0.09	3	3.18±---	0	0	0	0	0.50
		10	NS	432	1.74±0.05	12	2.44±0.01	0	0	0	0	2.70
	3/12/92	2	NS	1,570	2.56±0.3	29	3.43±0.17	0	0	0	0	1.81
		6	S	629	2.56±0.05	17	3.37±0.07	0	0	0	0	2.63
		10	NS	3,959	2.34±0.10	46	3.75±0.15	0	0	0	0	1.15
		11	NS	1,118	1.48±0.09	7	3.02±---	0	0	0	0	0.62
	3/29/92	4	NS	1,428	2.84±0.07	17	3.87±0.06	0	0	0	0	1.18
		10	NS	1,646	2.80±0.10	27	3.64±0.24	0	0	0	0	1.61
		18	NS	1,974	1.96±0.05	35	2.64±0.11	0	0	0	0	1.74
	7/22/92	29	NS	15,970	2.61±0.16	123	3.77±0.34	0	0	0	0	0.76

[1]Colony
[2]Sampling date
[3]Trap number
[4]Presence of stained (S) or absence of stained (NS) termites
[5]Number of workers
[6]Average worker weight (mg) (± SEM)
[7]Number of soldiers
[8]Average soldier weight (mg) (± SEM)

[9]Number of presoldiers
[10]Average presoldier weight (mg) (± SEM)
[11]Number of nymphs
[12]Average nymph weight (mg) (± SEM)
[13]Percentage of soldiers collected in each trap
[14]Austin Cary Memorial Forest
[15]Animal Science I
[16]Animal Science II
[17]Data not recorded

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BIOGRAPHICAL SKETCH

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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